Fungi associated with living woody tissue of sycamore maple (*Acer pseudoplatanus* L.) with special focus on sooty bark disease

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Contents

Abstract

The Sooty Bark Disease (SBD) is caused by the ascomycetous fungus *Cryptostroma corticale* which was first described in Canada from decaying wood in 1889 under the name *Coniothyrium corticale*. The first report of the disease from Europe dates back to1945, when it was reported from the UK, and shortly after from France. Since its first report from Europe the disease has caused periodic damage predominantly to *Acer pseudoplatanus* trees in most of the central European countries. The disease usually occurs more prominently after years with summer temperatures exceeding the recorded average. The latest outbreak in Germany started in 2018 and has slowed down in severity since 2021 but did not subside. The fungus causes symptoms like wilt, dieback, discolouration of the infected wood as well as death of the tree and finally the breaking open of the bark revealing the dark, sooty spore layer. Due to *Acer pseudoplatanus* (sycamore maple) being a promising tree species in regards to climate change, *e.g.* considering its high tolerance for high temperatures and frost, it is important to get a better understanding of the causal agent and the disease.

Considering the non-continuous occurrence of the disease the endophytic stage of the fungus, which had long been presumed, was investigated. In addition, fungi belonging to the mycobiome of the living woody tissue of *A. pseudoplatanus* were isolated and determined by analysis of the ITS sequences. Furthermore, it was investigated whether one of the fungi already known to be associated with *A. pseudoplatanus*, could be used as a potential biological control agent against *C. corticale*.

To achieve these goals five forest stands in Germany, in the federal states of Hesse and Schleswig-Holstein, with different damage levels in regard to SBD were chosen for sampling. Ten trees per forest stand were sampled by taking wood increments from visually healthy sycamore maple trees. The wood increments were surface sterilised and put on Petri dished filled with nutrient media in the mycological laboratory. *Cryptostroma corticale* was isolated from asymptomatic living woody tissue in the course of this study from three out of the five sites sampled, proving the endophytic stage of the fungus. As for the isolated fungi, 86.8% (n = 91 taxa) of the taxa belonged to the Ascomycota, while 9.9% of the taxa belonged to the Basidiomycota, and one isolate was classified as Mucoromycota. One isolate was classified as coelomycete, while another could only be identified as Fungus sp. The composition of the fungal community varied strongly among the different sites and even individual trees.

Four different in vitro antagonism assays were used to evaluate the antagonistic potential of 102 fungal isolates against *C. corticale*. In total, five fungi were identified to be potential antagonists against *C. corticale*, showing antagonistic behaviour in all tests. These are *Hypholoma fasciculare*, *Jackrogersella cohaerens*, *Paracamarosporium* cf. *fagi*, *Pezicula sporulosa*, and *Preussia* cf. *aemulans*. In future studies these fungi could be tested in *in vivo* trials to confirm their antagonistic properties against *C. corticale in vivo*.

Zusammenfassung

Die Rußrindenkrankheit, welche durch den Schlauchpilz (Ascomycet) *Cryptostroma corticale* ausgelöst wird, wurde zuerst in Kanada im Jahr 1889 beschrieben. Der Erreger wurde zunächst unter dem Namen *Coniothyrium corticale* beschrieben. Der erste Nachweis des Erregers und der Krankheit aus Europa stammt aus dem Jahr 1945 aus Großbritannien, kurze Zeit später wurden die Krankheit und der Erreger auch in Frankreich identifiziert. Seit der ersten Beobachtung in Europa tritt die Rußrindenkrankheit immer wieder periodisch in Folge auf über dem langjährigen Mittel liegende Sommertemperaturen auf. Das letzte großflächige Auftreten der Krankheit am Bergahorn (*Acer pseudoplatanus*) begann als Reaktion auf das besonders warme Jahr 2018. Das Pathogen verursacht Symptome wie Welke, Triebsterben, Verfärbung des befallenen Holzes sowie oftmals das Absterben des befallenen Baumes. Das wohl auffälligste Symptom ist die aufbrechende Rinde, unter welcher die rußartige Sporenschicht zu finden ist. Seine Sporen können über mehrere 100 km weit vom Wind verbreitet werden. Da Bergahorn eine vielversprechende Baumart im Hinblick auf den Klimawandel ist, welche gut mit beispielsweise hohen Sommertemperaturen sowie Frost zurechtkommt, ist es wichtig sowohl den Erreger als auch die Krankheit besser zu verstehen.

In Anbetracht des sporadischen Auftretens der Krankheit, wurde die endophytische Lebensphase von *C. corticale* untersucht. Hierfür wurden in fünf Waldbeständen mit Bergahorn in unterschiedlichen Schadstufen in Bezug auf die Rußrindenkrankheit, je zehn Bäume, welche augenscheinlich symptomlos und vital wirkten, untersucht. Es wurden Bohrkernproben genommen, welche im Labor oberflächensterilisiert und anschließend auf Petrischalen mit Nährmedium ausgelegt wurden. Die ausgewachsenen Pilze wurden nach Morphotypen gruppiert und anschließend basierend auf einer Analyse der ITS-Sequenzen bestimmt. Die Mehrheit (86.8 %, n = 91 Taxa) der isolierten Taxa gehörte dem Phylum der Schlauchpilze an, während ein deutlich geringerer Teil (9,9 %) dem Phylum der Ständerpilze (Basidiomycota) angehörte. Zusätzlich wurden ein Mucoromycota, ein Coelomyzet und ein Fungus sp. isoliert, die basierend auf der ITS Sequenz nicht genauer bestimmt werden konnten. Die Zusammensetzung der jeweils isolierten Pilzgemeinschaften unterschied sich stark zwischen den einzelnen Standorten, sowie auch zwischen den einzelnen Bäumen. *Cryptostroma corticale* wurde aus den Proben von drei der fünf Untersuchungsflächen aus symptomlosem Gewebe isoliert, wodurch die endophytische Lebensphase des Pilzes nachgewiesen werden konnte.

Anhand der aus Bergahornholz isolierten Pilze wurde untersucht, ob einer dieser Pilze als potentieller Antagonist gegen *C. corticale* in Frage kommen könnte. Mit vier verschiedenen *in vitro* Antagonistentests wurden die antagonistischen Fähigkeiten von 102 Isolaten untersucht. Basierend auf diesen Tests wurden fünf Pilze identifiziert, welche potenziell als biologische Kontrolle gegen *C. corticale* in Frage kommen könnten. Die als potenzielle Antagonisten in Frage kommenden Isolate sind *Hypholoma fasciculare*, *Jackrogersella cohaerens*, *Paracamarosporium* cf. *fagi*, *Pezicula sporulosa* und *Preussia* cf. *aemulans*. Diese Pilze könnten in weiterführenden Studien auch *in vivo* getestet werden, um auch hier die antagonistischen Fähigkeiten sicherzustellen.

1 General Introduction

1.1 Sycamore maple (*Acer pseudoplatanus* L.)

The genus *Acer* L. belongs to the family of the *Sapindaceae* and is currently grouped into 18 different sections, six of which are further classified into series. According to World Flora Online (2024; last accessed December 2024), 158 species (excluding subspecies and variations) for the genus *Acer* were listed as trees in the *Sapindaceae*. Most of the known *Acer* species are native to Asia, and the genus almost exclusively occurs in the northern hemisphere with only one exception, *Acer laurinum* Hassk. occurring in South-East Asia, including Malaysia and Indonesia (Feng 2008; Gibbs and Chen 2009). Three species naturally occur in German forests, namely *Acer campestre* L., *Acer platanoides* L., belonging to the section *Platanoidae* Pax, and *Acer pseudoplatanus* L. belonging to the Section *Acer* L. and Series *Acer* (Davis and The Maple Society 2021). Additional species in Germany can be found in urban areas. These include *Acer monspessulanum* L., *Acer negundo* L., and *Acer rubrum* L. (Vogt et al. 2017; Kowarik 2023; GALK 2025). *Acer* species account for 3% of German forests according to the fourth national forest inventory (BWI; bwi.info) from 2022.

The tree species *Acer pseudoplatanus* (commonly known as sycamore or sycamore maple, Figure 1) was first described in 1753 by Carl von Linné in the second volume of his work ‘Species Plantarum’ under the nomenclature we know today. The species is widely distributed and native to Europe and western Asia and has been introduced to North America, Australia and New Zealand, as well as Argentina and Chile (CABI 2022; World Flora Online 2024). *Acer pseudoplatanus* is able to colonise areas with high altitude, up to 1800 m (Schmidt and Roloff 2014; CABI 2022). The species requires good availability of nutrients in the soil and a good water supply, but is also known to be a pioneer species (Brosinger and Schmidt 2009). It shares its distribution range potential with common beech (*Fagus sylvatica* L.), common ash (*Fraxinus excelsior* L.), and wych elm (*Ulmus glabra* Huds) and is often found alongside common beech in Europe (Leuschner and Ellenberg 2017). In their early years, *Acer pseudoplatanus* trees are very fast growing and moderately shade tolerant. With time they become less shade tolerant and needs direct light. In competition with *F. sylvatica*, *A. pseudoplatanus* is competitive until its height growth culmination. The height growth of sycamore maple cumulates between the ages of four to 13 (Hein 2004). After the height growth has culminated beech overgrows sycamore maple, if sycamore maple is not given adequate space and light by active management. The average height for a mature sycamore maple tree is reached around age 80 at about 30 m (Hein 2004).

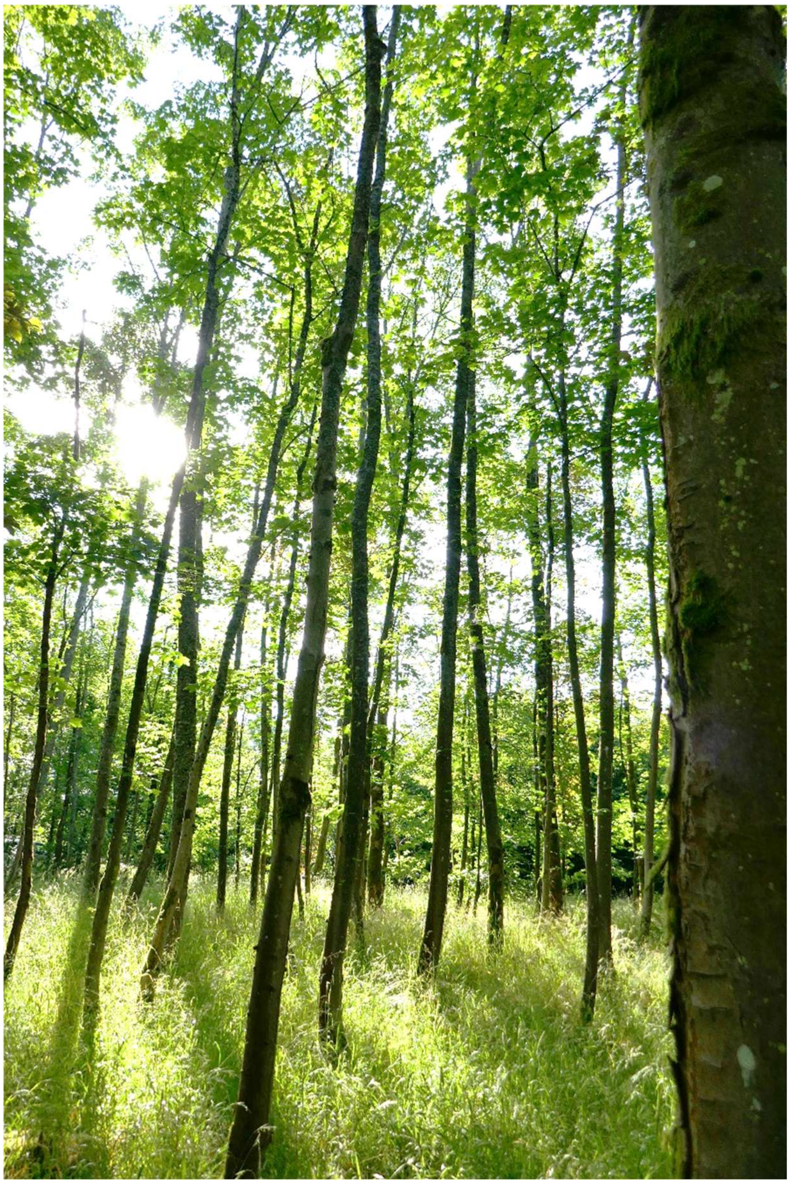

Figure 1: 55 year-old stand of *Acer pseudoplatanus* in Schotten, Hesse. Photo: Rebekka Schlößer.

Krabel and Wolf (2013) state that *A. pseudoplatanus* is the only European tree species that shows increasing vitality with increasing altitude. Since sycamore maple often grows together with common beech, the forest management has to be precise. The management strategy should aim for giving sycamore maple enough room and light to grow, otherwise it will be overgrown and outcompeted by common beech, which has a higher shade tolerance. Because of this, sycamore maple does not occur in pure stands in unmanaged forests in Germany. It most commonly occurs in mixed forest communities with other valuable timber species, like subalpine sycamore maple-beech forests (*Aceri-Fagion*) and lime-maple mixed forests (*Tilio-Acerion*; Runge 1994). Additionally, *A. pseudoplatanus* is widely used in urban areas as an ornamental tree species due to its high tolerance to *e.g.* pollution or salt winds, making it also suitable for coastal regions (Pasta et al. 2016). *Acer pseudoplatanus* has this far been considered to be one of the more promising tree species in respect of future forests in climate change due to *e.g.* its high tolerance for high temperatures as well as its frost tolerance (Schmidt and Roloff 2014; Avila et al. 2021).

Acer pseudoplatanus is considered a noble hardwood. Noble hardwoods are a group of species, including sycamore maple, common ash (*Fraxinus excelsior* L.), lime (*Tilia* sp. L.), and elm (*Ulmus* sp. L.; Krabel and Wolf 2013), which are extremely valuable for the German forest ecosystem because their presence, even if sporadic, highly increases both the commercial and ecological value of the forest (Brosinger and Schmidt 2009; Schmidt and Roloff 2014). Rożek et al. (2023), for example, report that in the soil under *A. pseudoplatanus* trees a high fungal species richness was found in comparison to the thirteen other tree species which were included in the study. Likewise, the species richness of bacteria found in the soil under the trees was highest under *A. pseudoplatanus* and *Tilia cordata* Mill. (Rożek et al. 2023), which also belongs to the group of the noble hardwoods. Furthermore, sycamore maple is an important host species for lichens in mountainous regions (Brosinger and Schmidt 2009).

Acer species are commonly colonized by pathogens in the phyllosphere, for example *Rhytisma acerinum* (Pers.) Fr., and *Petrakia echinata* (Peglion) Syd. & P. Syd. (Wulf et al. 2005). These leaf pathogens do not cause damage severe enough to have an influence on the growth of the trees though (Schwerdtfeger 1981; Kirisits 2007; Kehr and Wulf 2009). Only few fungal pathogens are known to cause symptoms, other than leaf spots, on sycamore maple. These include for example, *Eutypella parasitica* R.W. Davidson & R.C. Lorenz, which can cause cankers on the trunk of the trees (Wulf et al. 2005; Jurc et al. 2006; Brglez et al. 2020), *Nectria cinnabarina* (Tode) Fr., the causal agent of coral spot (Wulf et al. 2005; Kehr and Wulf 2009), *Stegonsporium pyriforme* (Hoffm.) Corda, which causes wilt symptoms and crown dieback and bark necrosis (Voglmayr and Jaklitsch 2008), and *Verticillium dahliae* Kleb. (Schneidewind 2005; Burgdorf et al. 2014) which can cause potentially lethal tracheomycosis on *A. pseudoplatanus*.

1.2 *Cryptostroma corticale*

Cryptostroma corticale (Ellis & Everh.) P.H. Greg. & S. Waller is the causal agent of the sooty bark disease (SBD; Ellis and Everhart, 1889; Gregory and Waller 1951). It was first described as *Coniosporium corticale* from Canada by Ellis and Everhart (1889). The species was later transferred to the monotypic genus *Cryptostroma* by Gregory and Waller (1951). *Cryptostroma corticale* is an ascomycete, presumably belonging to the *Graphostromataceae* M.E. Barr, J.D. Rogers & Y.M. Ju, Xylariales Tul. & C. Tul (Koukol et al. 2014). According to Koukol et al. (2014) the closest relatives to *C. corticale* are *Biscogniauxia bartholomaei* (Peck) Lar. N. Vassiljeva and *Graphostroma platystomum* (Schwein.) Piroz. The order of the Xylariales contains many fungi known to cause soft rot, including *C. corticale* (Worrall et al. 1997; Schwarze 2018). Contrary to several species belonging to the genus *Biscogniauxia*, no teleomorph has yet been identified for *C. corticale*.

The first report of *C. corticale* from Europe dates back to 1945, where it was identified from a park in London, UK (Gregory and Waller 1951). Not long after, in 1947, it was also found and identified in Paris (Moreau and Moreau 1951). As evident by the literature, reports of the SBD accumulate frequently after a dryer and warmer periods, as compared to the recorded temperature average over the years, for example around the 1950s and the 1970s (Moreau and Moreau 1951; Gregory and Waller 1951; Townrow 1953; Plate and Schneider 1965; Abbott et al. 1977; Abbey 1978; Young 1978; Dickenson 1980; Dickenson and Wheeler 1981; Abbey and Stretton 1985). Following the exceptionally warm years 2003 and 2005 (DWD 2004, 2005) more reports from several European countries were published (Cech 2004; Engesser et al. 2004; Metzler 2006; Robeck et al. 2008). Until now the SBD has been reported from Austria, Bulgaria, Belgium, Czech Republic, France, Germany, Italy, the Netherlands, Switzerland, and the United Kingdom (Gregory and Waller 1951; Plate and Schneider 1965; Cech 2004; Bencheva 2014; Koukol et al. 2014; Oliveira Longa et al. 2016; Muller et al. 2023). Recently, SBD has also been reported from the western part of Washington state (Brooks et al. 2023), and California, USA (Garbelotto et al. 2024), British Columbia, Canada (Tanney et al. 2024), as well as from Russia (Gninenko et al. 2024). Local outbreaks of SBD following the warm period of 2018-2020 (DWD 2018, 2019, 2020) in Germany were quite severe, as reported to the Northwest German Federal Research Institute (NW-FVA), causing entire forest stands to be cut down due to the amount of damaged and dead trees.

During the beginning of the latest large-scale outbreak of SBD in Germany, reports of SBD by foresters to the Northwest German Federal Research Institute (NW-FVA) often turned out to be *Stegonsporium pyriforme* instead of *C. corticale*. Both fungi produce black spores on *A. pseudoplatanus* (Figure 2). *C. corticale* produces a continuous dense spore layer between phellogen and phloem which is only revealed after the bark breaks open (Gregory and Waller 1951). *Stegonsporium pyriforme* forms ascocarps which penetrate the bark from the inside, forming non-continuous spots of sporulation on the bark (Voglmayr and Jaklitsch 2008).

Figure 2: left: the sooty spore layer of *C. corticale* on *A. pseudoplatanus*; right: for comparison regarding the differential diagnosis the black sporulation layer of *Stegonsporium pyriforme* on *A. pseudoplatanus*. Photos: Rebekka Schlößer.

Cryptostroma corticale is considered to be an opportunistic pathogen. It was been described as a saprotroph by Young (1978) and has been presumed to have an endophytic life stage (Gibbs 1998; Sieber 2007). The fungus infects trees through fresh wounds (Townrow 1953; Dickenson 1980) and can cause symptoms, including wilting of leaves and crown dieback in early stages, as well as a greenish to yellow discoloration of the infected wood, and later the production of black conidia in masses between phellogen and phloem (Gregory and Waller 1951; Pearce 2000; Enderle et al. 2020). Following Pearce (2000), the discoloration of the infected wood is a reaction zone caused by organic compounds involved in the host plant defense. Townrow

(1953) showed that the spores of *C. corticale* germinate on fresh and wet wounds of *A. pseudoplatanus* wood and then penetrate the tissue, as opposed to being sucked into the vessels. *Cryptostroma corticale* appears to prefer colonizing non-lignified tissue within the plant (Townrow 1953). The spore walls of *C. corticale* are lined with melanin, which makes them resistant to heat and direct sunlight (Gregory and Waller 1951). The main mode of dispersal of the spores is through wind and rainsplash. At low temperatures the spores survive for up to 2 years, without losing the ability to germinate (Dickenson 1980). It has been shown by Kelnarová et al. (2017) that *C. corticale* can be detected in 25% of all sycamore maples examined in their study, regardless of health status. Similar results were obtained by Lorenc (2024). The spores of *C. corticale* can be transported for large distances with the wind of at least 310 km (Muller et al. 2023). Infections over large distances in previously unaffected areas are not only possible but likely due to its mode of spread and characteristics.

Recently, it could be confirmed that *C. corticale* is able to infect, and in some cases damage, other species outside of the genus *Acer*. Young (1978) reported *C. corticale* as saprophyte on *Aesculus hippocastanum* L. Recent reports from Germany confirmed that *C. corticale* can also cause symptoms on *A. hippocastanum* (Brenken et al. 2024), while recent reports from Italy show the endophytic occurrence of *C. corticale* in *A. hippocastanum* (Schlößer et al. 2024). Another recent report shows the presence of *C. corticale* also in *F. excelsior* (Langer et al. 2024). *Cryptostroma corticale* was re-isolated from further tree species following an *in vivo* infection study including *Euonimus europaeus* L., *Populus tacamahaca* x *trichocarpa*, *Salix viminalis* L. and *Viburnum opulus* L (Dickenson 1980). Trials of sporulation on autoclaved wood pieces which were placed onto growth media covered by mycelium of *C. corticale* were carried out by Dickenson (1980). Her experiment showed, that *C. corticale* is able to sporulate of several *Acer* species as well as on *A. hippocastanum*, *E. europaeus*, *Fagus sylvatica*, *Ligustrum vulgare* L., *Platanus* x *hispanica*, *Platanus orientalis* L., *Populus nigra* L., *Prunus spinosa* L., *Quercus robur* L., *Salix caprea* L., *S. viminalis*, *Taxus baccata* L., and *Tilia* x *europaea* L.

1.3 Mycobiome of trees and endophytes of trees

The entirety of the fungal community associated with plants is called the mycobiome. The composition of the fungi varies strongly between individual trees, tree species, different types of plant tissue, as well as the location of the plant (*e.g.* Hoffman and Arnold 2008). It is well known that all trees host a large community of fungi, part of which aid the host plant in terms of fitness, health, *i.e.* against stress and pathogens, and nutrition (*e.g.* Vandenkoornhuyse et al. 2015; Aswani et al. 2022). Fungi can colonise plants both on the tissue surface (epiphytes) as well as inside the tissue (endophytes).

The definition of endophytic fungi has been changed and adjusted throughout the years. Johann Heinrich Friedrich Link was the first researcher to describe fungi being present in plant material without causing harm in 1809 (Adeleke and Babalola 2021; Rabbee et al. 2024). The term 'endophyte' was first used by Anton de Bary (1866) and directly translates to "inside the plant". De Bary (1866) included all organisms living inside the plant in his definition of the term 'endophyte' (de Bary 1866). Currently, the most common understanding is that endophytic fungi are fungi that spend a significant part of their life cycle within living host plant tissue without causing symptoms (Petrini 1991; Saikkonen et al. 1998; Arnold and Lutzoni 2007; Sieber 2007). Two main groups of endophytic fungi are recognized. These are clavicipitalean and non-clavicipitalean endophytes (Carroll 1988; Petrini 1991; Stone and Petrini 1997; Stone et al. 2004; Schulz and Boyle 2005). The clavicipitalean endophytes (C-endophytes, Class 1), belonging to the family of the *Clavicipitales*, Ascomycota, have a narrow host range, only colonizing grasses, while the non-clavicipitalean endophytes (NC-endophytes, divided into Class 2-4), belonging to families other than the *Clavicipitales*, which are comprised predominantly of Ascomycota and to a lesser extent also of Basidiomycota and have a broader host range (Rodriguez et al. 2009). C-endophytes are transmitted vertically, whereas NC-endophytes, in general, can be transmitted both vertically and horizontally (Kehr 1998; Rodriguez et al. 2009). Within the NC-endophytes, the fungi are divided into three classes according to *e.g.* host range, fitness benefits to the host, or colonized plant tissue (Rodriguez et al. 2009). Endophytes belonging to Class 2 are capable of protecting their host against fungal pathogens (Danielsen and Jensen 1999; Campanile et al. 2007), for example by fungal parasitism of the potential pathogen (Samuels et al. 2000), induction of systemic host resistance (Vu et al. 2006) or the production of secondary metabolites which can be antifungal or induce host defense mechanisms (Schulz et al. 1999). They can be found inside both above and below ground tissue of plants (Rodriguez et al. 2009). In addition, Class 2 endophytes can improve a plant's stress tolerance, and some are known to be mutualists, conferring positive fitness benefits and acquiring nutrients from their hosts for their own benefit (Rodriguez et al. 2004, 2009). Redman et al. (1999) showed that cucurbit species, like watermelon and squash, in symbiosis with a non-virulent strain of a pathogen, in this case *Colletotrichum magna* (S.F. Jenkins & Winstead) Bhairi, E.P. Buckley & R.C. Staples, displayed the ability to activate their defense mechanisms earlier than non-symbiotic plants. Additionally, Rodriguez et al. (2009) state that plants colonized with Class 2 endophytes do not activate their host defense in the absence of pathogen exposure. Class 3 endophytes occur primarily, some exclusively, in above-ground tissue and have a vast biodiversity *in planta* (Rodriguez et al. 2009). It is not possible to isolate Class 3 endophytes from plants grown under sterile conditions, showing they are transmitted horizontally (Rodriguez et al. 2009). They are able to form highly localized infections and are not strictly habitat specific (Rodriguez et al. 2009; Terhonen et al. 2019). Class 3 endophytes can reproduce through hyphal fragmentation as well as production of spores on dead tissue (Herre et al. 2005 as cited in Rodriguez et al. 2009). Class 4 endophytes are dark septate endophytes, which are restricted to the rhizosphere (Rodriguez et al. 2009). This group of endophytes is generally not host or habitat specific, and is most likely transmitted horizontally (Rodriguez et al. 2009; Terhonen et al. 2019).

Sieber (2007) studied endophytes in leaves, wood and bark of several different tree species. According to Sieber (2007) *Pleosporales* and *Xylariales* play a large role in the fungal communities associated with angiosperms, while the fungal communities in *Aceraceae* are primarily dominated by fungal species belonging to the *Diapothales*. The fungal community of living tissue of *Acer pseudoplatanus* specifically, has this far not been researched vigorously. Only a few publications have analysed the fungal communities of living tissue of sycamore maple (Ellis and Ellis 1985; Kowalski and Kehr 1992; Schlegel et al. 2018; Lorenc 2024; Bußkamp et al. 2024). Several other studies exist referring to dead tissue of sycamore maple (Butin and Kowalski 1986; Chlebicki 1988; Unterseher et al. 2005; Brglez et al. 2020). In these studies, the most commonly isolated orders were *Diaporthales*, *Helotiales*, *Hypocreales,* and *Pleosporales*. *Diaporthales* did not unequivocally dominate in any the previously cited studies.

1.4 Biosecurity, biological control and bioprotection

Due to the increasing international trade and subsequent unintended and undetected introductions of organisms into new regions (Pautasso et al. 2010; Santini et al. 2013; Hulme 2021) many National Plant Protection Organisations (NPPOs), following the guidelines of the European Plant Protection Organisation (EPPO; EU 2019) have implemented phytosanitary control measures. Such measures are in place for *e.g. Ceratocystis platani* (Ministero delle Politiche Agricole Alimentari e Forestali 2015) in Italy or *Agrilus planipennis* Fairmaire in

Germany (EU 2019, EU 2019/2072). The aim is to prevent the introduction of non-native species, which might cause substantial damage to native tree species. This has previously happened in the case of *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya causing ash dieback in Europe (Gross et al. 2014; Mckinney et al. 2014) or Myrtle rust caused by *Austropuccinia psidii* (G. Winter) Beenken in Australia and New Zealand (Carnegie et al. 2010; Large and Galbraith 2017).

For phytosanitary measures to be put in place, a potential pathogen has to be already known and previously been identified as a threat. In many cases imported species that became invasive were not previously known to be a potential threat and were thus not included legal regulations. Based on the definition by the German Federal Agency of Nature Conservation (BfN), an invasive species is a non-native species which endangers the biological diversity of the area of introduction and is competing with native species for resources and habitat as well as convey diseases or influence the genepool of native species (BfN 2025). Today, approaches exist to identify potential pathogens before events of introduction (Raffa et al. 2023), for example sentinel plants and sentinel experimental plantings. Sentinel plantings can be established as *in-patria* or *ex-patria* plantings. *In-patria* sentinel plantings include plants native to both the importing and the exporting country, respectively, which are scanned for potential pathogens without being protected against them (Eschen et al. 2019; Migliorini et al. 2023). *Ex-patria* sentinel plantings are comprised of non-native plants from an importing country planted in an exporting country, to prevent the export of organisms potentially dangerous to native species in the importing country (Eschen et al. 2019). Other methods applied after potential introductions include early detection using molecular tools, such as loop-mediated isothermal amplification (LAMP) or qPCR (*e.g.* Hamelin and Roe 2020; Luchi et al. 2020), both of which require species-specific primers. A qPCR-assay has for example been developed for *Ceratocystis platani* (Luchi et al. 2013) and an assay for LAMP detection has been established for *e.g. Fusarium circinatum* Nirenberg & O'Donnell (Fourrier et al. 2015). The project “Biosurveillance of Alien forest enemies“, for example, aims to quickly establish early detection genetic tools for the detection of forest invasive pathogens of high priority (Hamelin and Roe 2020). To employ these methods the target organism has to already be known or suspected. Even though these methods exist, they are not yet being used extensively enough to detect all known imported pathogens. Furthermore, the kingdom of fungi is one of the most unexplored and there are estimated to be many more species yet undiscovered (Hawksworth and Rossman 1997; Niskanen et al. 2023; Hyde et al. 2024). If the scanning process to detect an invasive pathogen fails, measures of containment or eradication should be executed. If a pathogen becomes established to the point where it cannot be eradicated, biological control could be an option to aid in species survival of the host plants and disease pressure.

Biological control commonly refers to living organisms aiding in the protection against a pest at the benefit of humans (Stenberg et al. 2021), while bioprotection is a broader term encompassing all forms of nature-based control options (International Biocontrol Manufacturers Association 2025), for example fungal antagonists or secondary metabolites. Most of the published research referring to biocontrol is carried out in agricultural settings due to the short life span of crops in comparison to perennial plants as well as the economic importance of agricultural production (Pratt et al. 1999). Over the years, three different approaches regarding biological control have emerged. Namely, classical biological control, augmentation biological control and conservative biological control (Kenis et al. 2019; Prospero et al. 2021). Classical biological control refers to the introduction of a non-native natural enemy against a non-native pathogen to achieve a balance in the population of the pathogen, keeping it at a supportable level (Kenis et al. 2019). Augmentation biological control is executed by periodically releasing a biological control agent (BCA), to increase its density and thus effectiveness in control (Kenis et al. 2019). Conservative biological control is defined as “manipulation of habitat with the aim of enhancing the reproduction, survival and efficacy of natural enemies already present in the affected area” (Kenis et al. 2019).

Antagonism between fungi has been studied for many years. The antagonistic properties of fungi belonging to the genus *Trichoderma* against plant pathogens were described as early as the 1930s (Howell 2003). Boddy (2000) stated that the outcome of fungal interactions in antagonism assays can be either classified as replacement or deadlock. A switch from one to the other interaction type can occur sometimes. An antagonistic reaction can be caused by several different mechanisms, though multiple mechanisms can work simultaneously. These mechanisms include antibiosis, myco- and hypoparasitism, induced resistance and growth enhancement as well as competition for nutrients or substrate (Heydari and Pessarakli 2010; Busby et al. 2016; Thambugala et al. 2020). In the context of biological control an interaction is classified as mycoparasitism when the antagonist derives nutrients from its host, the pathogen. The best-known example of a hypoparasite being used in biological control in forestry is *Cryphonectria hypovirus* 1 (CHV1) which is used for control against *Cryphonectria* *parasitica* (Murr.) Barr. infecting *Castanea* spp. (Heiniger and Rigling 1994; Rigling and Prospero 2018). When infected with CHV1, the sporulation capacity as well as the parasitic growth of *C. parasitica* are reduced, thus not causing damage as severe in the host plant (Rigling and Prospero 2018). Some organisms are able to induce host resistance against certain pathogens (Conrath et al. 2001, 2006). For example, *Brassica napus* L. plants colonised by *Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W. Gams activate their host defense in response to colonisation with *Plasmodiophora brassicae* Woronin (Lahlali and Peng 2014). Another common mechanism in antagonism is competition between organisms for both nutrients and substrate (Boddy 2016). In this case, a potential antagonist should colonize the tissue prior to the arrival of the pathogen, or utilize the same nutrients in a more efficient manner. Holdenrieder and Greig (1998) for example, showed this for *Phlebiopsis gigantea* (Fr.) Jülich as a control against *Heterobasidion annosum* s.l. (Fr.) Bref. on spruce stumps. Lastly, there is the induction of host plant resistance, which is achieved by *e.g.* either priming with low-virulence strains of the pathogen or endophytes, as well as secondary metabolites (Bonello et al. 2001; Eyles et al. 2010; Rabiey et al. 2019; Thambugala et al. 2020). One example of induced host resistance is the infection with endophytes which induce the production of salicylic acid and jasmonic acid, both of which trigger the immune signalling network of the plant, as for example demonstrated for infections by *Ophiostoma novo-ulmi* Brasier in *Ulmus minor* Mill. (Caarls et al. 2015; Martínez-Arias et al. 2021). In antibiosis the antagonistic organism produces inhibitory organic compounds with antibiotic properties against the pathogen. The growth of a pathogen can be suppressed by antibiotics both *in vitro* and *in vivo* (Heydari and Pessarakli 2010 and literature cited herein). In some cases antibiosis can even lead to the death of the pathogen (Heydari and Pessarakli 2010). One common type of organic compounds with antibiotic properties are secondary metabolites.

Secondary metabolites are low weight compounds produced by an organism (Sanchez and Demain 2011; Seyedsayamdost 2019). Unlike primary metabolites, secondary metabolites are a by-product of the metabolism (Nawrot-Chorabik et al. 2022). Production of secondary metabolites by organisms is strongly dependent on biotic, as well as abiotic factors such as temperature, pH, and humidity (Nielsen et al. 2004; Keller et al. 2005; Qaderi et al. 2023). Secondary metabolites can be classified into various groups based on chemical structure and functional groups (Teoh 2016; Reshi et al. 2023): terpenes (including volatile compounds, sterols, and carotenoids), polysaccharides, phenolic compounds, phytoalexins (sulfurcontaining compounds), alkaloids (nitrogen-containing compounds), flavonoids, and hydrocarbons. These organic compounds can have direct impacts on the host plants as well as other organisms (Reshi et al. 2023). Some of these compounds have antibiotic or antimicrobial properties, while others are *e.g.* colour pigments. The properties of organic compounds are diverse and some have more than one property (Chen et al. 2022). In some antagonistic interactions following mycelial contact, a non-self recognition can be observed (Figure 3). This reaction is visible through a ‘barrage’, which is caused by a cell death reaction following the non-self recognition (Glass and Dementhon 2006; Paoletti et al. 2007), and often pigments are produced during this interaction (Boddy 2016). A non-self recognition results in both fungi stopping their growth in the direction of their opponent.

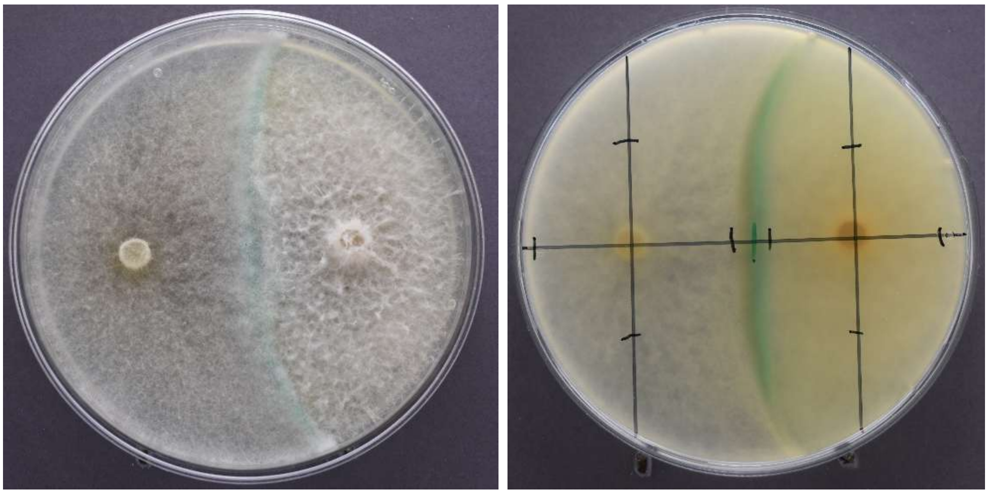

Figure 3: Upside and downside of a dual-culture antagonism assay showing non-self recognition between *C. corticale* (left on the plate) and *Apiospora* cf. *marii* (right on the plate) with blue/ greenish pigmentation in the interaction zone. Photo: Rebekka Schlößer.

Another example involved in host resistance are phytoalexins, which are produced by plants as a reaction to newly detected infections, and thus are relevant to defense against pathogens (Hammerschmidt and Kuć 1995). Hormones exuded by some microbes can have growth enhancing effects on the host plant while simultaneously reducing the impact and severity of a present disease caused by a pathogen (Van Wees et al. 2008). This can result in the plant's defense system responding more quickly when a pathogen is detected (Van Wees et al. 2008). Growth enhancing effects were for example observed for isolates of *Trichoderma* on *Brassica napus* L. (Zhao et al. 2022).

1.5 Objectives of the thesis

Even though *Cryptostroma corticale* has been known for being present in Europe since the 1940s (Moreau and Moreau 1951; Gregory and Waller 1951) very little research had been done on the fungus. The aim of this study was to gain a better understanding of the dispersion of the fungus in German forests with a particular focus on Hesse and Schleswig-Holstein as well as to gain a better understanding of the mycobiome of living woody tissue of *A. pseudoplatanus*. The author investigated whether *C. corticale* has a latent endophytic occurrence and which other fungi can be associated with living woody tissue of *A. pseudoplatanus.* It was further investigated, whether one of the isolated taxa has the potential to control the spread of *C. corticale*. The focus of the sampling was the federal state of Hesse in central western Germany, with four sampling sites in different regions and each with a different health status concerning the sooty bark disease. One additional site in Schleswig-Holstein, the northernmost federal state of Germany, was sampled as well since no outbreak of SBD had been reported at the time of sampling from forests in this federal state. Subsequently, due to the lack of a present control against *C. corticale*, it was investigated whether one of the fungi isolated from the woody tissue of *A. pseudoplatanus* could potentially function as an antagonist against *C. corticale*.

2 Manuscript I

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ORIGINAL ARTICLE

Fungi associated with woody tissues of *Acer pseudoplatanus* in forest stands with different health status concerning sooty bark disease (*Cryptostroma corticale*)

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Abstract
From 2018 to 2020, Germany experienced periods of exceptional weather conditions. Extremely high summer temperatures and precipitation deficits induced stress and mortality in forest trees. *Acer pseudoplatanus* (sycamore) was one of the affected tree species. Symptoms of sooty bark disease (SBD) and severe damage of entire stands, both caused by the fungal species *Cryptostroma corticale*, were reported more frequently. To explore the non-symptomatic distribution of *C. corticale*, wood cores from visibly healthy sycamore stems were sampled and all outgrowing fungi were identified and recorded. In total, 50 trees, aged 30–65 years, were sampled at five different forest stands, from which 91 endophytic filamentous morphotypes could be isolated. The fungal endophytic community in the woody tissue of the sycamore trees varied greatly at the different sites and between the trees. The number of isolated morphotypes at the different sites ranged from 13 to 44 and no morphotype was found at all sites. At 1.20-m stem height, 3.3 fungi could be isolated from woody tissue per tree on average. The most abundant species isolated from visibly healthy sycamore in regard to both occurrence at the studied sites and continuity was *C. corticale*. It was recorded at four of the studied forest stands, from 26% of all studied sycamore trees, and had a frequency of 7.85% relative to the 293 isolated filamentous strains that were isolated. The second most abundant species was *Xylaria longipes* followed by *Lopadostoma turgidum*. In this study clear evidence for the endophytic lifestyle of *C. corticale* is presented which thus appears to be spread further than expected based on visible SBD symptoms.

Keywords *Acer pseudoplatanus* · *Cryptostroma corticale* · Endophytic fungi · Fungal community · Sooty bark disease

Introduction

Sycamore (*Acer pseudoplatanus* L., *Sapindaceae*) is a deciduous tree that can be found throughout large parts of Europe (EUFORGEN 2022). In Germany, besides *A. pseudoplatanus*, two other native *Acer* species occur, namely *Acer platanoides* L. and *Acer campestre* L. Pure maple stands, if found in Germany, are the result of active management, since no natural pure *Acer* stands exist in this region. According to the phytosociological assignment and syntaxonomical treatment following Runge (1994), maple occurs in subalpine sycamore-beech forests (*Aceri-Fagion*) or in lime-maple mixed forests (*Tilio-Acerion*). In German forests, maple is often found in mixed stands, on calcareous soil with good nutrient and good water supply. *Acer pseudoplatanus* can be found in various forest communities, mainly paired with beech and highly valuable hardwoods (Schmidt 2009). The timber is very durable and thus often used for furniture and floors. Additionally, the occasionally wavy grained wood is used for the manufacture of musical instruments, making sycamore a valuable timber species (EUFORGEN 2022). Sycamore is a very valuable urban tree species as well.

Even though many fungi are reported from *A. pseudoplatanus* (244 different fungal genera according to the USDA website, Farr and Rossman (2022)), only a few studies focus specifically on endophytes of sycamore or fungi associated with the living woody tissue of sycamore (Kowalski and Kehr 1992; Kelnarová et al. 2017). The available data on fungi associated with *A. pseudoplatanus* mainly refers to dead wood (Butin and

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Kowalski 1986; Chlebicki 1988; Unterseher et al. 2005; Brglez et al. 2020a) or to leaves (Schlegel et al. 2018).

According to Petrini (1991), Saikkonen et al. (1998), Arnold and Lutzoni (2007), and Sieber (2007), we consider those fungi as endophytes that spend a significant amount of their life cycle within the host plant tissue without causing any symptoms there. A change of environmental conditions can cause a change in the lifestyle of the fungus from endophytic to pathogenic (Sieber 2007) or to saprotrophic when the host tissue dies (Sun et al. 2011). Some fungi can be endophytic in one tree species and pathogenic in another tree species (Petrini 1991; Saikkonen et al. 2010; Sanz-Ros et al. 2015). In other cases, endophytes can change to a pathogenic lifestyle under the right circumstances, such as stress in the host tree and high summer temperatures (Ragazzi et al. 2003; Hyde and Soytong 2008). Many wood-decay fungi appear to have a transient endophytic lifestyle, which may be in preparation for their saprotrophic life stage (Boddy and Rayner 1983; Parfitt et al. 2010).

As a result of the very warm and dry summers and mild winters in the years 2018–2020, many forest trees started showing signs of stress and mortality. In Germany, these years were characterised by mean daily temperatures during the meteorological summer months (June, July, and August) averaging from 19.3 °C in 2018 (2.2 °C above average of the reference period from 1981 to 2010), to 19.2 °C in 2019 (+2.1 °C above average) and 18.2 °C in 2020 (+1.1 °C above average (DWD 2018, 2019, 2020)). The precipitation sum for the three summer months was 130 l/m^2 in 2018, 175 l/m^2 in 2019, and 230 l/m^2 in 2020, while the required average precipitation for the reference period is 239 l/m^2 (DWD 2018, 2019, 2020). As a consequence, German forests developed a soil water deficit resulting in signs of stress in the trees (NW-FVA 2020, 2021). Following these weather extremes, an extraordinary outbreak of sooty bark disease (SBD), caused by the invasive fungus *Cryptostroma corticale* (Ellis & Everh.) P.H. Greg. & S. Waller (Ellis and Everhart, 1889; Gregory and Waller 1951), was observed more frequently in several regions in Germany (Bork 2018; Rohde et al. 2019; Wenzel et al. 2019; Delb et al. 2019). Symptoms included wilting and dieback in the crown in earlier stages, as well as the production of masses of black conidia under the outer layer of the bark in later stages of the disease (Enderle et al. 2020; Schlößer and Langer 2021).

Cryptostroma corticale is presumed to be opportunistic with endophytic, pathogenic, and saprophytic life stages that react to stress, and it is known to have an optimal growing temperature of 25 °C (Dickenson 1980; Enderle et al. 2020). Currently, there is no published evidence for an endophytic/latent lifestyle of the pathogen, despite occasional isolations of *C. corticale* from symptomless tissue (e.g. Kelnarová et al. (2017); Tropf (2020)). However, in these cases, the symptomless tissue samples originate from trees already showing SBD symptoms (wood discolouration, defoliation, etc.) in other parts of the tree. As the distance of investigated symptomless samples to symptomatic tissue in no study is communicated, the fungal growth could have simply extended beyond the recognizable necrotic tissue; and therefore, a true endophytic/latent phase expressed by this species is questionable. It is assumed that *C. corticale* primarily infects the tree through fresh wounds (Townrow 1953; Dickenson 1980) and causes a soft rot in infested tissues of the tree, like many other closely related fungi from the *Xylariales* (Worrall et al. 1997; Schwarze 2018). The original description of *C. corticale* by Ellis and Everhart (1889) originated from Canada. The first record in Germany reported by the plant protection office in Berlin dates back to 1964 (Plate and Schneider 1965). In this case, spores of *C. corticale* were detected on infested firewood originating from trees of the Berlin Tiergarten park area and stored in a basement. Triggered by extremely warm weather and precipitation deficits in the year 2003, several incidences of SBD occurred in this and the following years throughout Germany and Europe (Cech 2004; Engesser et al. 2004; Metzler 2006; Robeck et al. 2008; Langer et al. 2013; Bencheva 2014; Koukol et al. 2014). The recently observed cases of disease from 2018 to 2021 constitute the biggest outbreaks of SBD in Germany yet. Since the data are recorded by each forest protection office in Germany for their respective regions, there is a gap in compiled data about the actual distribution of SBD in German forests. A preliminary distribution map regarding German forests was published by Schlößer and Langer (2021); the updated version is presented in this paper.

The main goals of this research were to undertake a better assessment of (1) fungi associated with woody tissues of *Acer pseudoplatanus* paired with an investigation into the spread of *C. corticale* in its endophytic stage, and (2) the current status and distribution of *C. corticale* as well as the potential risks of SBD in Germany. Therefore, the distribution of SBD in German forests was mapped based on compiled data from forest protection offices in Germany and fungi associated with living sycamore woody stem tissue from forest stands with different health status in respect to SBD were studied. In order to explore fungi associated with living woody tissue of sycamore, as well as the non-symptomatic distribution of *C. corticale* in visibly healthy trees, a study was conducted examining stem wood cores, following an adjusted version of the method used by Kelnarová et al. (2017). The results of this study are relevant regarding the risk assessment of potential disease outbreaks especially in light of the ongoing climate change.

Materials and methods

Mapping of the sooty bark disease

In order to map the distribution of SBD cases in German forests, three different approaches were perused: (1) infestation data from the federal forest protection institutions of

Germany were compiled (deadline 31.05.2021); (2) from May 2020 to September 2020, 123 forest stands with *A. pseudoplatanus* (aged 20–100 years, with sycamore as a dominant tree species) in Hesse were evaluated with regard to visible symptoms of sooty bark disease; (3) data on cases of SBD in Schleswig-Holstein, Lower Saxony, Hesse, and Saxony-Anhalt were retrieved from the 'Waldschutzmeldeportal' Forest Protection Reporting Portal (WSMP) of the Northwest German Forest Research Institute (NW-FVA); and (4) cases of SBD directly reported by forest owners or foresters and checked by the authors between May 2020 and March 2022. Forest owners and foresters can register georeferenced forest damage in the online WSMP. QGIS (v. 3.22.3, www.qgis.org) was used to create a combined map based on a preliminary distribution map (Schlößer and Langer 2021).

Sampling sites

Wood samples were taken from five forest stands (Table 1) containing sycamore, located in two federal states of Germany. Four of the sites are located in Hesse in the middle of Germany and one, Nehmten, in Schleswig-Holstein, in the very north of Germany. The four Hessian sites were located in the forest departments of Melsungen, Nidda, Fulda, and Beerfelden. Two of the five stands, in Fulda and in Nidda, were visibly affected by SBD at the time of sampling, and several maple trees exhibited black conidia underneath the ruptured bark. In the studied stands in Melsungen, Beerfelden, and Nehmten, no visible signs of SBD were observed. The forest stands without symptoms of SBD were located at different distances from the nearest known infestation point with *C. corticale*. The studied site in Beerfelden was 200 m away from the next SBD-affected trees, while in Melsungen there was more than 30 km of aerial distance to the closest infested stand. In Schleswig-Holstein, no case of SBD in forests had been reported until June 2022. Forest stands of different health status were chosen in order to check for occurrence of *C. corticale* in stands without visible signs of SBD.

Isolation of fungi

At each site, ten living and obviously healthy trees, aged 30–65 years and without visible symptoms of SBD, were sampled using increment borers (Haglöf Increment Borer Mora-Coretax, three-edged, 300 mm drilling depth, 5.15 mm diameter). Two 20 cm increments of stem tissue per tree were taken at a 1.20 m stem height above ground following the method of Kelnarová et al. (2017). In Melsungen, the first sampled site, three increments were taken per tree. The bark of the sample trees was sprayed with 70% ethanol at sampling height and wiped with a paper towel. Two borers were used alternately

Table 1 Sampling site conditions; apart from Nehmten (mesotrophic), all other studied forest sites are eutrophic. *UTM*, Universal Transverse Mercator (Grid)

Forest site (coordinates UTM)	Date of sampling	Elevation above sea level (m)	Exposition	Inclination	Climate	Soil water supply	Bedrock	Stand tree components	Visible signs of SBD	*C. corticale* isolated
Beerfelden (32 U 485720 5492792)	09.09–10.09.2021	450–500	North and south	Moderately inclined (0°–18°)	Strongly subatlantic	Slightly moist/moist	Granite with loamy loess	Maple, sycamore, European beech	No	Yes
Fulda (32 U 546727 5586419)	04.05.2021	410–450	West	Moderately inclined (0°–18°)	Weakly subatlantic	Slightly moist/moist	Basalt with loamy loess	Hornbeam, European beech, maple	Yes	No
Melsungen (32 U 539938 5674066)	10.03.2021	470–490	Hilltop	Slightly inclined (0°–9°)	Moderately subatlantic	Moist/changing water content	Basalt with loamy loess	Maple, European beech, European ash	No	Yes
Nehmten (32 U 589629 5993467)	30.09.–31.09.2021	66	South-west	Moderate inclination (0°–9°)	Subatlantic	Moist/high water storing capacity	Diluvial sand. Boulder clay	Sycamore	No	Yes
Nidda (32 U 502557 5581999)	13.07.2021	290–330	North	Moderate to slight inclination (0°–18°)	Weakly subatlantic	Slightly dry/ slightly moist	Basalt	European ash, sycamore	Yes	Yes

and sterilised by flame shortly before every use. The outer layer of the bark was carefully scraped and the exposed tissue was disinfected with ethanol again. Increments, taken on the vertical axis, were carefully removed from the extractor by disinfected hands and placed into clean and marked plastic tubes. The borer, extractor, and knife were cleaned each time before taking a new sample by spraying with ethanol and wiping away the excess plant material which was followed by flame sterilisation. Samples were cooled during transportation to the laboratory. Sampling was performed from March to September 2021. For each tree, diameter at breast height (1.30 m) as well as the following were recorded: crown vitality, the number of epicormic shoots as an indicator for vitality as well as visible signs of infestation with *C. corticale* or *Stegonsporium pyriforme* (Hoffm.) Corda (Online Resource 1).

In the laboratory, each increment was surface sterilised by rinsing the sample with 70% ethanol and left to dry for a few minutes. Increments were cut into 5 mm-long segments and three pieces were placed on each 90 mm petri dish containing malt yeast peptone (MYP) agar, modified according to Langer (1994) containing 0.7% malt extract (Merck, Darmstadt, Germany), 0.05% yeast extract (Fluka, Seelze, Germany), 0.1% peptone (Merck, Darmstadt, Germany), and 1.5% agar (Fluka, Seelze, Germany). From the sampling sites in Fulda, Beerfelden, Nidda, and Nehmten, the increment segments with visible wood discolouration or signs of rot were observed and counted. The petri dishes were incubated at room temperature with ambient daylight for 4 weeks. The cultures were monitored every second day in the first 2 weeks and twice a week in the third and fourth weeks. Emerging mycelia were sub-cultured into pure cultures. The pure cultures were tentatively grouped into morphotypes (MTs) based on morphological observation following the method of Schulthess and Faeth (1998). At least one representative culture for each MT was stored on MYP slants at 4 °C in the fungal culture collection of the NW-FVA.

Frequency of isolated taxa, defined as proportion of isolated strains in relation to the total number of isolated filamentous strains, was calculated. Continuity of isolated taxa, defined as the number of trees from which the fungus was isolated in relation to the total number of trees, was calculated.

Molecular analysis

As a rule, one representative strain per MT was used for genetic analysis and species identification. Mycelium was placed in 1.5 ml Eppendorf tubes with five glass beads (3 mm) and 150 μl of TE buffer (10 ml 1 mmol Tris HCl (pH 0.8), 2 ml 0.5 mmol EDTA; Carl Roth, Karlsruhe, Germany). The mycelium was crushed in a Mixer Mill MM 200 (Retsch, Haan, Germany) with 25 vibrations per second for 90 s. Subsequently, genomic DNA was extracted following the protocol of Izumitsu et al. (2012).

For all strains, the 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers ITS-1 and ITS-2 (ITS) was amplified using the primer pair ITS-1F (Gardes and Bruns 1993) + ITS-4 (White et al. 1990). For strains belonging to *Neonectria*, the actin gene (*ACT*) and portion of the β-tubulin gene (*TUB*) were additionally amplified using primer pairs T*act*1 + T*act*2 (Samuels et al. 2006) and T1 (O'Donnell and Cigelnik 1997) + Bt-2b (Glass and Donaldson 1995), respectively. The PCR mixture consisted of 1 μl of DNA and 19 μl mastermix which contained 2.5 μl 10× PCR reaction buffer (with 20 mM $MgCl_2$, Carl Roth, Karlsruhe, Germany), 1 μl of each primer (10 mmol), 2.5 μl $MgCl_2$ (25 mmol), 0.1 μl Roti®-Pol Taq HY Taq polymerase (Carl Roth, Karlsruhe, Germany), and 2.5 μl of 2 mmol dNTPs (Biozym Scientific GmbH, Hessisch Oldendorf, Germany). Each reaction was topped up to a volume of 20 μl by adding sterile water.

A StepOnePlus™ PCR System (Applied Biosystems, Waltham, Massachusetts, USA) was used to carry out the DNA amplifications. The PCR conditions for the amplification of the ITS region were set according to Bien et al. (2020). The amplification conditions for the primer pair T*act*1 + T*act*2 were as follows: initial denaturation at 94 °C for 10 min; followed by 30 cycles of denaturation at 94 °C for 35 s, annealing at 48 °C for 30 s and extension at 72 °C for 80 s; and a final extension step of 10 min at 72 °C. The amplification conditions for the primer pair T1 + Bt-2b were set according to Cabral et al. (2012b) with the exception of a 60 °C annealing temperature. A 1% agarose gel was used to visualise the PCR products. The products were sent to Eurofins Scientific Laboratory (Ebersberg, Germany) for sequencing. Initially, PCR samples of the ITS region were sequenced using the forward reaction (primer ITS-1F). In case of imprecise results, reverse reactions (primer ITS-4) were sequenced in addition. All other DNA sequence regions were sequenced by the respective forward and reverse reactions. By using BioEdit Sequence Alignment Editor (v. 7.2.5; Hall (1999)), all sequences were visually checked, and defective sequence beginnings and ends trimmed. In case of forward and reverse sequences available, consensus sequences were generated using BioEdit and further processed in the same way. Sequences were submitted to GenBank (Table 2).

Identification of fungi

Morphotypes were assigned to a taxonomic level by molecular analysis of representative strains of each morphotypic group following the method of Guo et al. (2000). The BLAST algorithm (http://www.ncbi.nlm.nih.gov/genbank, Altschul et al. (1997)) was used for fungal taxon determination. The results were re-checked against literature and known cultures for confirmation. BLAST results below a threshold of 98% identity were not trusted to be accurate enough for final

Table 2 List of isolated fungi sorted alphabetically within division: A, *Ascomycota*; B, *Basidiomycota*; M, *Mucoromycota*; I.s., Incertae sedis; occurrence at sites (Melsungen, Fulda, Beerfelden, Nidda, and Nehmten): 0, not isolated; 1, isolated. Basis of identification: '-' indicates that no NCBI Blast result was a close match for identification or that DNA extraction was unsuccessful. Blast conducted: 21.02.2022

Species	Division	Order	NW-FVA ID	Accession No.[1]	n isolates	Frequency (%)	Continuity (%)	Sites isolated from
cf. *Akanthomyces*	A	Hypocreales	6247	-	1	0.34	2	1
Angustimassarina sp.	A	*Pleosporales*	6253	ON710906^{I}	5	1.71	4	2
Apiognomonia sp.	A	*Diaporthales*	6272	ON710918^{I}	1	0.34	2	1
Arthrinium cf. *marii*	A	I.s.	7004	ON710953^{I}	5	1.71	4	1
Arthrinium rasikravindrae	A	I.s.	6999	ON710949^{I}	4	1.37	6	1
Arthrinium sp.	A	I.s.	7713	-	1	0.34	2	1
Aureobasidium sp.	A	*Dothideales*	6586	ON710936^{I}	1	0.34	2	1
Beauveria cf. *bassiana*	A	*Hypocreales*	6581	ON710933^{I}	1	0.34	2	1
Biscogniauxia nummularia	A	*Xylariales*	6227	ON710888^{I}	5	1.71	6	2
Boeremia exigua	A	*Pleosporales*	6430	ON710928^{I}	1	0.34	2	1
Cadophora prunicola	A	*Helotiales*	6596	ON710939^{I}	3	1.02	6	3
Calosporella innesii	A	*Diaporthales*	6573	ON786729^{I}	3	1.02	2	1
Capronia sp.	A	*Chaetothyriales*	6262	ON710913^{I}	2	0.68	4	1
Cladosporium sp. 1	A	*Capnodiales*	6597	ON710940^{I}	2	0.68	4	2
Cladosporium sp. 2	A	*Capnodiales*	7195	ON710963^{I}	1	0.34	2	1
Cladosporium sp. 3	A	*Capnodiales*	6259	ON710910^{I}	1	0.34	2	1
Cladosporium sp. 4	A	*Capnodiales*	6598	ON710941^{I}	2	0.68	2	1
Cladosporium sp. 5	A	*Capnodiales*	6991	ON710944^{I}	1	0.34	2	1
Cladosporium sphaerospermum	A	*Capnodiales*	7706	ON710965^{I}	1	0.34	2	1
Clonostachys rosea	A	*Hypocreales*	6243	ON710898^{I}	1	0.34	2	1
Cryptostroma corticale	A	*Xylariales*	7001	ON710951^{I}	23	7.85	26	4
Cytospora cf. *populina*	A	*Diaporthales*	6237	ON710895^{I}	19	6.48	8	1
Cytospora cf. *rodophila*	A	*Diaporthales*	6232	ON710892^{I}	15	5.12	8	1
Cytospora sp.	A	*Diaporthales*	6249	ON710902^{I}	1	0.34	2	1
Diaporthe cf. *eres*	A	*Diaporthales*	7714	ON710970^{I}	1	0.34	2	1
Diaporthe cf. *rudis*	A	*Diaporthales*	7002	ON710952^{I}	4	1.37	4	1
Diaporthe pustulata	A	*Diaporthales*	6228	ON710889^{I}	3	1.02	6	2
Didymella macrostoma	A	*Pleosporales*	6244	ON710899^{I}	6	2.05	6	2
Didymellaceae sp.	A	*Pleosporales*	6998	ON710948^{I}	1	0.34	2	1
Dothideomycetes sp.	A	I.s.	6429	ON710927^{I}	1	0.34	2	1
Dothiorella spp.	A	*Botryosphaeriales*	6230	ON710891^{I}	1	0.34	2	1
Eutypa cf. *petrakii* var. *hederae*	A	*Xylariales*	6267	ON710915^{I}	1	0.34	2	1
Eutypa maura	A	*Xylariales*	6245	ON710900^{I}	6	2.05	4	1
Eutypella quaternata	A	*Xylariales*	6238	ON710896^{I}	3	1.02	4	1
Exophiala cf. *pisciphila*	A	*Chaetothyriales*	6423	ON710924^{I}	1	0.34	2	1
Furcasterigmium furcatum	A	*Glomerellales*	7014	ON710955^{I}	1	0.34	2	1
Hypoxylon fragiforme	A	*Xylariales*	7707	ON710966^{I}	2	0.68	4	1
Hypoxylon rubiginosum	A	*Xylariales*	7711	ON710968^{I}	1	0.34	2	1
Jackrogersella cohaerens	A	*Xylariales*	7020	ON710957^{I}	1	0.34	2	1
cf. *Leptodontidium* sp.	A	*Helotiales*	6276	ON710921^{I}	1	0.34	2	1
Leptosillia muelleri	A	*Xylariales*	6576	ON710931^{I}	6	2.05	12	2
Lopadostoma turgidum	A	*Xylariales*	6575	ON710930^{I}	10	3.41	10	3
Lophiostoma carpini	A	*Pleosporales*	6993	ON710946^{I}	2	0.68	2	1
Lophium arboricola	A	*Mytilinidiales*	6260	ON710911^{I}	1	0.34	2	1

Table 2 (continued)

Species	Division	Order	NW-FVA ID	Accession No.[1]	n isolates	Frequency (%)	Continuity (%)	Sites isolated from
Melanomma populicola	A	*Pleosporales*	6274	ON710920^{I}	1	0.34	2	1
Mycoarthris sp.	A	*Helotiales*	6426	ON710925^{I}	1	0.34	2	1
Nectria cinnabarina	A	*Hypocreales*	6236	ON710894^{I}	9	3.07	4	1
Neocucurbitaria acerina	A	*Pleosporales*	6258	ON710909^{I}	2	0.68	4	1
Neodidymelliopsis sp.	A	*Pleosporales*	6261	ON710912^{I}	2	0.68	4	2
Neoleptosphaeria rubefaciens	A	*Pleosporales*	6255	ON710907^{I}	1	0.34	2	1
Neonectria cf. *ramulariae*	A	*Hypocreales*	6264	ON710914^{I} ON803475^{Act} ON803478^{T}	3	0.68	4	1
Neonectria punicea	A	*Hypocreales*	7203	ON710964^{I} ON803476^{Act} ON803479^{T}	2	0.68	2	1
Neonectria sp.	A	*Hypocreales*	6582	ON710934^{I} ON803477^{Act} ON803480^{T}	1	0.34	2	1
Neosetophoma cf. *italica*	A	*Pleosporales*	6250	ON710903^{I}	4	1.37	6	1
Neosetophoma cf. *samarorum*	A	*Pleosporales*	6252	ON710905^{I}	2	1.02	2	1
Nigrograna cf. *norvegica*	A	*Pleosporales*	7189	ON710960^{I}	1	0.34	2	1
Nigrograna mycophila	A	*Pleosporales*	6990	ON710943^{I}	1	0.34	2	1
Paracamarosporium cf. *fagi*	A	*Pleosporales*	6420	ON710923^{I}	1	0.34	2	1
Penicillium cf. *tularense*	A	*Eurotiales*	7021	ON710958^{I}	1	0.34	2	1
Penicillium sp.	A	*Eurotiales*	6231	-	9	3.07	12	1
Petrakia irregularis	A	*Pleosporales*	6580	ON710932^{I}	2	0.68	2	1
Pezicula sporulosa	A	*Helotiales*	6992	ON710945^{I}	1	0.34	2	1
Phoma sp.	A	*Pleosporales*	6248	ON710901^{I}	6	2.05	6	1
Pleosporales sp. 1	A	*Pleosporales*	6278	ON710922^{I}	1	0.34	2	1
Pleosporales sp. 2	A	*Pleosporales*	6989	ON710942^{I}	1	0.34	2	1
Pleosporales sp., cf. *Splanchnonema*	A	*Pleosporales*	6590	ON710937^{I}	1	0.34	2	1
Preussia cf. *aemulans*	A	*Pleosporales*	6585	ON710935^{I}	1	0.34	2	1
Pseudogymnoascus cf. *pannorum*	A	*Thelebolales*	6428	ON710926^{I}	1	0.34	2	1
Querciphoma carteri	A	*Pleosporales*	6594	ON710938^{I}	1	0.34	2	1
Ramularia collo-cygni	A	*Mycosphaerellales*	7023	ON710959^{I}	1	0.34	2	1
Sordariomycetes sp. 1	A		7712	ON710969^{I}	1	0.34	2	1
Sordariomycetes sp. 2	A		6572	ON710929^{I}	1	0.34	2	1
cf. *Thyridiaceae*	A	*Thyridiales*	6256	ON710908^{I}	2	0.68	2	1
Thyridium vestitum	A	*Thyridiales*	6251	ON710904^{I}	14	4.78	8	1
Tolypocladium sp.	A	*Hypocreales*	7191	ON710961^{I}	1	0.34	2	1
cf. *Tolypocladium* sp.	A	*Hypocreales*	7010	ON710954^{I}	1	0.34	2	1
Trichoderma sp.	A	*Hypocreales*	7705	-	3	1.02	6	3
Xenocylindrosporium sp.	A	I.s.	6592	ON786728^{I}	1	0.34	2	1
Xylaria longipes	A	*Xylariales*	6229	ON710890^{I}	9	3.07	14	4
Agaricales sp.	B	*Agaricales*	7192	ON710962^{I}	2	0.68	4	1
Coprinellus micaceus	B	*Agaricales*	6233	ON710893^{I}	2	0.68	4	1
Coprinellus sp.	B	*Agaricales*	7000	ON710950^{I}	1	0.34	2	1

Table 2 (continued)

Species	Division	Order	NW-FVA ID	Accession No.^{1}	n isolates	Frequency (%)	Continuity (%)	Sites isolated from
Hymenochaetaceae sp.	B	*Hymenochaetales*	7019	ON710956^{I}	31	10.58	4	2
Hypholoma fasciculare	B	*Agaricales*	6241	ON710897^{I}	2	0.68	2	1
Porostereum spadiceum	B	*Polyporales*	6997	ON710947^{I}	1	0.34	2	1
Serpula himantioides	B	*Boletales*	7710	ON710967^{I}	1	0.34	2	1
Stereum cf. *hirsutum*	B	*Russulales*	6270	ON710916^{I}	1	0.34	2	1
Trametes versicolor	B	*Polyporales*	6271	ON710917^{I}	3	1.02	6	1
Mucoromycota sp.	M		7022	-	1	0.34	2	1
coelomycete			7709	-	1	0.34	2	1
Fungus sp.			7196	-	2	0.68	4	2

Species	Sampling sites					ITS NCBI Blast results			
	Melsungen	Fulda	Beerfelden	Nidda	Nehmten	Basis of identification	ID	Identity (%)	Reference
cf. *Akanthomyces*	1	0	0	0	0	DNA extraction failed	-	-	-
Angustimassarina sp.	1	1	0	0	0	*Lophiostoma corticola*	KU712227.1	99.46	Langer (2017)
Apiognomonia sp.	1	0	0	0	0	*Apiognomonia pseudohystrix*	MT177938.1	97.81	Li et al. (2020)
Arthrinium cf. *marii*	0	0	1	0	0	*Arthrinium marii*	NR_166043.1	99.82	Vu et al. (2019)
Arthrinium rasikravindrae	0	0	1	0	0	*Arthrinium rasikravindrae*	MT487807.1	100	Limsivilai and Yurayart unpublished
Arthrinium sp.	0	0	0	0	1	morphologically determined	-	-	-
Aureobasidium sp.	0	1	0	0	0	*Aureobasidium* sp.	MN378414.1	100	Bueno et al. (2020)
Beauveria cf. *bassiana*	0	1	0	0	0	*Beauveria bassiana*	MH859798.1	98.82	Vu et al. (2019)
Biscogniauxia nummularia	1	0	0	1	0	*Biscogniauxia nummularia*	MH860015.1	100	Vu et al. (2019)
Boeremia exigua	1	0	0	0	0	*Boeremia exigua*	MN077467.1	100	Johnston unpublished
Cadophora prunicola	0	1	1	1	0	*Cadophora prunicola*	MN232951.1	100	Bien and Damm (2020a)
Calosporella innesii	0	1	0	0	0	*Prosthecium innesii*	JF681965.1	100	Kruys and Castlebury 2012
Capronia sp.	1	0	0	0	0	*Capronia pulcherrima*	AF050256.1	99.15	Untereiner and Naveau (1999)
Cladosporium sp. 1	0	1	0	0	1	*Cladosporium allicinum*	MT573471.1	100	Wysoczański et al. (2021)
Cladosporium sp. 2	0	0	0	1	0	*Cladosporium herbarum*	MH864860.1	100	Vu et al. (2019)
Cladosporium sp. 3	1	0	0	0	0	*Cladosporium perangustum*	MT645919.1	100	Cambon et al. unpublished
Cladosporium sp. 4	0	1	0	0	0	*Cladosporium* sp.	LC586224.1	99.80	Itagaki and Hosoya (2021)
Cladosporium sp. 5	0	0	1	0	0	*Cladosporium* sp.	MN853869.1	100	Haenzi et al. (2021)
Cladosporium sphaerospermum	0	0	0	0	1	*Cladosporium sphaerospermum*	MF473261.1	99.80	Bensch et al. (2018)
Clonostachys rosea	1	0	0	0	0	*Clonostachys rosea*	MH865141.1	100	Vu et al. (2019)
Cryptostroma corticale	1	0	1	1	1	*Cryptostroma corticale*	MH857008.1	100	Vu et al. (2019)
Cytospora cf. *populina*	1	0	0	0	0	*Valsa ambiens*	MH862828.1	100	Vu et al. (2019)
Cytospora cf. *rodophila*	1	0	0	0	0	*Cytospora rhodophila*	KY051928.1	100	Jami et al. Unpublished

Table 2 (continued)

Species	Sampling sites					ITS NCBI Blast results			
	Melsungen	Fulda	Beerfelden	Nidda	Nehmten	Basis of identification	ID	Identity (%)	Reference
Cytospora sp.	1	0	0	0	0	*Cytospora* sp.	KU516449.1	100	Jankowiak (2005)
Diaporthe cf. *eres*	0	0	0	0	1	*Diaporthe eres*	MK442579.1	99.09	Crous et al. (2019)
Diaporthe cf. *rudis*	0	0	1	1	0	*Diaporthe rudis*	KC343232.1	100	Gomes et al. (2013)
Diaporthe pustulata	1	1	0	0	0	*Diaporthe pustulata*	KC343187.1	100	Gomes et al. (2013)
Didymella macrostoma	1	1	0	0	0	*Didymella macrostroma*	MH858090.1	99.58	Vu et al. (2019)
Didymellaceae sp.	0	0	1	0	0	*Didymella pomorum*	MH861278.1	100	Vu et al. (2019)
Dothideomycetes sp.	1	0	0	0	0	*Nematostoma parasiticum*	MT547819.1	97.04	Bilanski et al. unpublished
Dothiorella spp.	1	0	0	0	0	*Dothiorella vimadera*	MT587416	100	Zhang et al. 2021
Eutypa cf. *petrakii* var. *hederae*	1	0	0	0	0	*Eutypa* cf. *petrakii* var. *hederae*	MH862077	99.46	Vu et al. (2019)
Eutypa maura	1	0	0	0	0	*Eutypa maura*	AY684224.1	97.65	Trouillas and Gubler (2004)
Eutypella quaternata	1	0	0	0	0	*Eutypella quaternata*	MN698987.1	99.84	Bußkamp and Langer unpublished
Exophiala cf. *pisciphila*	1	0	0	0	0	*Exophiala pisciphila*	MH859072.1	99.84	Vu et al. (2019)
Furcasterigmium furcatum	0	0	1	0	0	*Furcasterigmium furcatum*	MH859660.1	99.8	Vu et al. (2019)
Hypoxylon fragiforme	0	0	0	0	1	*Hypoxylon fragiforme*	MH855287.1	100	Vu et al. (2019)
Hypoxylon rubiginosum	0	0	0	0	1	*Hypoxylon rubiginosum*	KC968929.1	98.04	Kuhnert et al. (2014)
Jackrogersella cohaerens	0	0	0	1	0	*Annulohypoxylon cohaerens*	KU516435.1	99.81	Jankowiak et al. (2016)
cf. *Leptodontidium* sp.	1	0	0	0	0	*Leptodontidium elatius*	AY787713.2	100	Lygis et al. (2005)
Leptosillia muelleri	0	1	1	0	0	*Leptosillia muelleri*	NR_164065.1	100	Voglmayr et al. (2019)
Lopadostoma turgidum	0	1	1	1	0	*Lopadostoma turgidum*	KC774617.1	98.21	Jaklitsch et al. (2014)
Lophiostoma carpini	0	0	1	0	0	*Lophiostoma carpini*	NR_173000.1	99.80	Andreasen et al. (2021)
Lophium arboricola	1	0	0	0	0	*Lophium arboricola*	NR_153447.1	99.39	Bills et al. (1999)
Melanomma populicola	1	0	0	0	0	*Melanomma populicola*	MT223816.1	100	Crous et al. (2020)
Mycoarthris sp.	1	0	0	0	0	*Mycoarthris* sp.	MZ493003.1	100	Kowalski and Bilański (2021)
Nectria cinnabarina	1	0	0	0	0	*Nectria cinnabarina*	MH856245.1	100	Vu et al. (2019)
Neocucurbitaria acerina	1	0	0	0	0	*Neocucurbitaria acerina*	NR_154254.1	98.91	Wanasinghe et al. (2017)
Neodidymelliopsis sp.	1	1	0	0	0	*Neodidymelliopsis polemonii*	NR_158233.1	98.56	Aveskamp et al. (2010)
Neoleptosphaeria rubefaciens	1	0	0	0	0	*Neoleptosphaeria rubefaciens*	MT153724.1	100	Bien and Damm (2020b)
Neonectria cf. *ramulariae*	1	0	0	0	0	*Neonectria candida*	JF735314.1	99.82	Cabral et al., 2012a)
Neonectria punicea	0	0	1	0	0	*Neonectria faginata*	HQ840385.1	100	Zhao et al. (2011)
Neonectria sp.	0	1	0	0	0	*Neonectria candida*	OK338557.1	100	Dove et al. unpublished
Neosetophoma cf. *italica*	1	0	0	0	0	*Neosetophoma italica*	LC206635.1	99.5	Hosoya et al. (2018)
Neosetophoma cf. *samarorum*	1	0	0	0	0	*Neosetophoma samarorum*	KF251162.1	98.42	Quaedvlieg et al. (2013)

Table 2 (continued)

Species	Sampling sites					ITS NCBI Blast results			
	Melsungen	**Fulda**	**Beerfelden**	**Nidda**	**Nehmten**	**Basis of identification**	**ID**	**Identity (%)**	**Reference**
Nigrograna cf. *norvegica*	0	0	0	1	0	*Nigrograna norvegica*	NR_147655.1	97.87	Jaklitsch and Voglmayr (2016)
Nigrograna mycophila	0	0	1	0	0	*Nigrograna mycophila*	NR_147654.1	98.11	Jaklitsch and Voglmayr (2016)
Paracamarosporium cf. *fagi*	1	0	0	0	0	*Paracamarosporium fagi*	NR_154318.1	100	Crous et al. (2015)
Penicillium cf. *tularense*	0	0	0	1	0	*Penicillium tularense*	JX313166.1	94.85	Frisvad et al. (2013)
Penicillium sp.	1	0	0	0	0	DNA extraction failed	-	-	-
Petrakia irregularis	0	1	0	0	0	*Petrakia irregularis*	NR_164281.1	99.60	Vu et al. (2019)
Pezicula sporulosa	0	0	1	0	0	*Pezicula sporulosa*	MH862573.1	100	Vu et al. (2019)
Phoma sp.	1	0	0	0	0	*Phoma* sp.	MK066907.1	100	Bußkamp et al. (2020)
Pleosporales sp. 1	1	0	0	0	0	*Pleosporales* sp.	MH063651.1	97.81	Glynou et al. (2018)
Pleosporales sp. 2	0	0	1	0	0	*Parapyrenochaeta* sp.	MK441755.1	98.13	Liu and Xu unpublished
Pleosporales sp., cf. *Splanchnonema*	0	1	0	0	0	*Splanchnonema pupula*	MN251065	100	Brglez et al., 2020a)
Preussia cf. *aemulans*	0	1	0	0	0	*Preussia aemulans*	MH858745.1	99.59	Vu et al. (2019)
Pseudogymnoascus cf. *pannorum*	1	0	0	0	0	*Pseudogymnoascus pannorum*	MH864775.1	100	Vu et al. (2019)
Querciphoma carteri	0	1	0	0	0	*Querciphoma carteri*	KF251209.1	100	Quaedvlieg et al. (2013)
Ramularia collo-cygni	0	0	0	1	0	*Ramularia collo-cygni*	NR_154944.1	100	Videira et al. (2016)
Sordariomycetes sp. 1	0	0	0	0	1	*Phomatospora biseriata*	NR_154640.1	98.69	Senanayake et al. (2016)
Sordariomycetes sp. 2	0	1	0	0	0	*Sordariomycetes* sp.	KR909162.1	98.14	Travadon et al. (2015)
cf. *Thyridiaceae*	1	0	0	0	0	*Parathyridaria* sp.	MN244551.1	100	Brglez et al. (2020b)
Thyridium vestitum	1	0	0	0	0	*Thyridium vestitum*	MH863721.1	99.82	Vu et al. (2019)
Tolypocladium sp.	0	0	0	1	0	*Tolypocladium* sp.	MT153684.1	99.81	Bien and Damm (2020b)
cf. *Tolypocladium* sp.	0	0	1	0	0	*Tolypocladium* sp.	MW064161.1	98.00	Utermann et al. (2020)
Trichoderma sp.	1	1	0	0	1	DNA extraction failed	-	-	-
Xenocylindrosporium sp.	0	1	0	0		*Xenocylindrosporium* sp.	MT791078.1	98.79	Spies et al. (2020)
Xylaria longipes	1	1	0	1	1	*Xylaria longipes*	MG098261.1	100	Bußkamp et al. (2020)
Agaricales sp.	0	0	0	1	0	*Hemipholiota populnea*	MG735315.1	99.85	Matheny et al. unpublished
Coprinellus micaceus	1	0	0	0	0	*Coprinellus micaceus*	KU712252.1	100	Langer (2017)
Coprinellus sp.	0	0	1	0	0	*Coprinellus ellisii*	MH858016.1	100	Vu et al. (2019)
Hymenochaetaceae sp.	0	0	0	1	1	*Inonotus cuticularis*	KF446595.1	93.31	Zhou et al. (2014)
Hypholoma fasciculare	1	0	0	0	0	*Hypholoma fasciculare*	MZ493083.1	100	Kowalski and Bilański (2021)
Porostereum spadiceum	0	0	1	0	0	*Porostereum spadiceum*	MH856439.1	98.15	Vu et al. (2019)
Serpula himantioides	0	0	0	0	1	*Serpula himantioides*	MH855789	99.3	Vu et al. (2019)
Stereum cf. *hirsutum*	1	0	0	0	0	*Stereum hirsutum*	LN714607.1	99.84	Větrovský et al. (2016)

Table 2 (continued)

Species	Sampling sites					ITS NCBI Blast results			
	Melsungen	Fulda	Beerfelden	Nidda	Nehmten	Basis of identification	ID	Identity (%)	Reference
Trametes versicolor	1	0	0	0	0	*Trametes versicolor*	AM084699.1	99.49	Anderson and Parkin (2007)
Mucoromycota sp.	0	0	0	1	0	DNA extraction failed	-	-	-
coelomycete	0	0	0	0	1	DNA extraction failed	-	-	-
Fungus sp.	0	0	1	1	0	DNA extraction failed	-	-	-

[1] *I*, internal transcribed spacers and intervening 5.8S nrDNA; *T*, β-tubulin gene; *Act*, actin gene

determination. Each identification was critically interpreted with emphasis on well-curated culture collections such as the Westerdijk Fungal Biodiversity Collection (CBS). In case no definite affiliation was possible to a specific taxonomic level, the identification was marked by cf. (confer) to indicate uncertainties.

Since BLAST results for different *Neonectria* and *Eutypa* strains were inconclusive, additional comprehensive analyses were performed. For strains belonging to *Neonectria*, final determination of the genus and species was based on two separate comprehensive phylogenetic analyses using the *ACT* and *TUB* gene regions, respectively, including reference sequences retrieved from GenBank (data not shown). Similarly, for the final determination of different *Eutypa* strains, a phylogenetic analysis based on the ITS results was accomplished.

Analysis with R

Analysis of the fungal diversity found in this study was conducted using RStudio V 4.1.2 (R Core Team 2021). The package tidyverse (Wickham et al. 2019) was used, where a distribution chart was created using the function 'pie'. The packages ggplot 2 (Wickham 2016) and ggVennDiagram (Gao 2021) were used to analyse the overlap between the fungi found at each site. Here the function 'ggVennDiagram' was used.

It was further checked manually and using RStudio (R Core Team 2021) whether the presence of *C. corticale* influenced the fungal community using the 'plot' function as well as the 'ddply' function of the plyr package (Wickham 2011) and a distance matrix using the function 'vegdist' from the package vegan (Oksanen et al. 2022) was generated. Additionally, it was analysed whether the third increment taken at Melsungen had a significant influence on the diversity of isolated morphotypes at that site using the 'plot' function as well.

Results

Distribution of SBD in German forests

By 31.05.2021, we had received reports of SBD from forests all over Germany with the exception of the federal state of Schleswig-Holstein, where no outbreak has been recorded so far. In total, 403 stands with obvious symptoms of SBD were reported (Fig. 1). Of the 123 evaluated stands in Hesse, 31.71% showed visible symptoms of SBD. At the beginning of 2022, a new collection of SBD reports were registered in the WSMP for Hesse and added to the map.

Isolated fungi

In total, 4124 segments of stem tissue increments originating from 50 sycamore trees were incubated. From these, 379

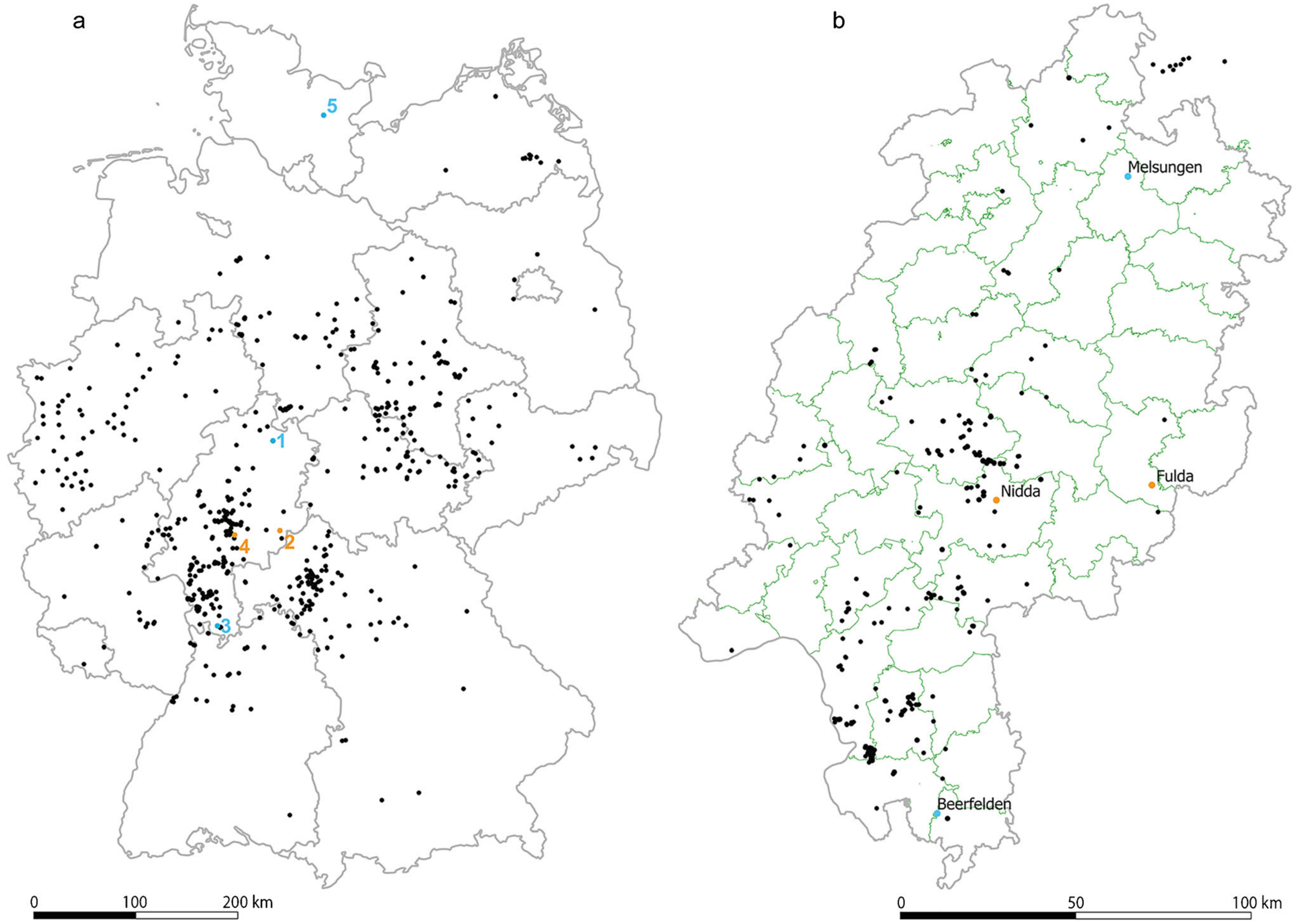

Fig. 1 **a**: Distribution of SBD cases in Germany, reported by 31.05.2021, last updated for Hesse: 31.03.2022, 1 = Melsungen, 2 = Fulda, 3 = Beerfelden, 4 = Nidda, 5 = Nehmten; **b**: detailed view of the cases in the federal state of Hesse; © GeoBasis-DE / BKG (2021), © HessenForst 2020, last updated: 31.03.2022 (QGIS Desktop 3.22.3); ● sampling sites without sooty bark disease, ● sampling sites with sooty bark disease, ● reported stands with visible sooty bark disease, ▭ hessian forest department borders, ▭ borders of the federal states.

mycelial outgrows were observed and 292 of them were transferred to pure cultures (Online Resource 2). Most fungi grew out between weeks 1 and 3 after incubation of the increment segment. When the original plates were discarded, only a few outgrowths had been observed in the preceding week. Some of the 379 outgrows were omitted due to obvious repetitions or contaminations. The resulting pure culture isolates were assigned to 91 morphotypes and all but 14 could be assigned to genus or species level (Table 2, Fig. 2). The majority of the wood tissue increments studied (93.8%, n = 3163 studied increment segments) had no visible discolouration or even signs of rotting. From sampling sites in Fulda, Beerfelden, Nidda, and Nehmten, 28 of the 80 increments showed visible signs of infection, resulting in 195 increment segments with wood discolouration or rot (6.2%, n = 3163). From those segments, yeast grew out in 78 cases and mycelia in 43 cases (nine morphotypes), and no growth was observed in 78 cases. Seven of the isolated morphotypes from discoloured or decayed wood were assigned to genus or species level, namely *Biscogniauxia nummularia* (Bull.) Kuntze, *C. corticale*, *Diaporthe* sp., *Furcasterigmium furcatum* (C. Moreau & Moreau ex W. Gams) Giraldo López & Crous, *Jackrogersella cohaerens* (Pers.) L. Wendt, Kuhnert & M. Stadler, *Leptosillia muelleri* (Duby) Voglmayr & Jaklitsch, and *Neonectria* sp. The remaining two morphotypes could be assigned to the order of *Agaricales* (NW-FVA 7192) and the family of *Hymenochaetaceae* (cf. *Inonotus* sp. NW-FVA 7019), respectively.

The majority of the isolated filamentous fungi from all samples were *Ascomycota* (79 taxa, 86.81%), nine taxa (9.89%) belonged to the division of *Basidiomycota*, and one morphotype was determined to be *Mucoromycota* (1.1%). The remaining two taxa (2.19%) could not be classified due to unsuccessful DNA extraction, one presumably being a coelomycete fungus. Within the *Ascomycota*, the most frequently observed orders (Fig. 3) were *Pleosporales* (26.58%), followed by *Xylariales* (13.92%) and *Hypocreales* (12.66%). The *Basidiomycota* morphotypes were assigned to *Agaricales* sp. (NW-FVA 7192), *Coprinellus micaceus* (Bull.) Vilgalys, Hopple & Jacq. Johnson, *Coprinellus* sp. (NW-FVA 7000), *Hymenochaetaceae* sp. (NW-FVA 7019), *Hypholoma fasciculare* (Huds.) P.

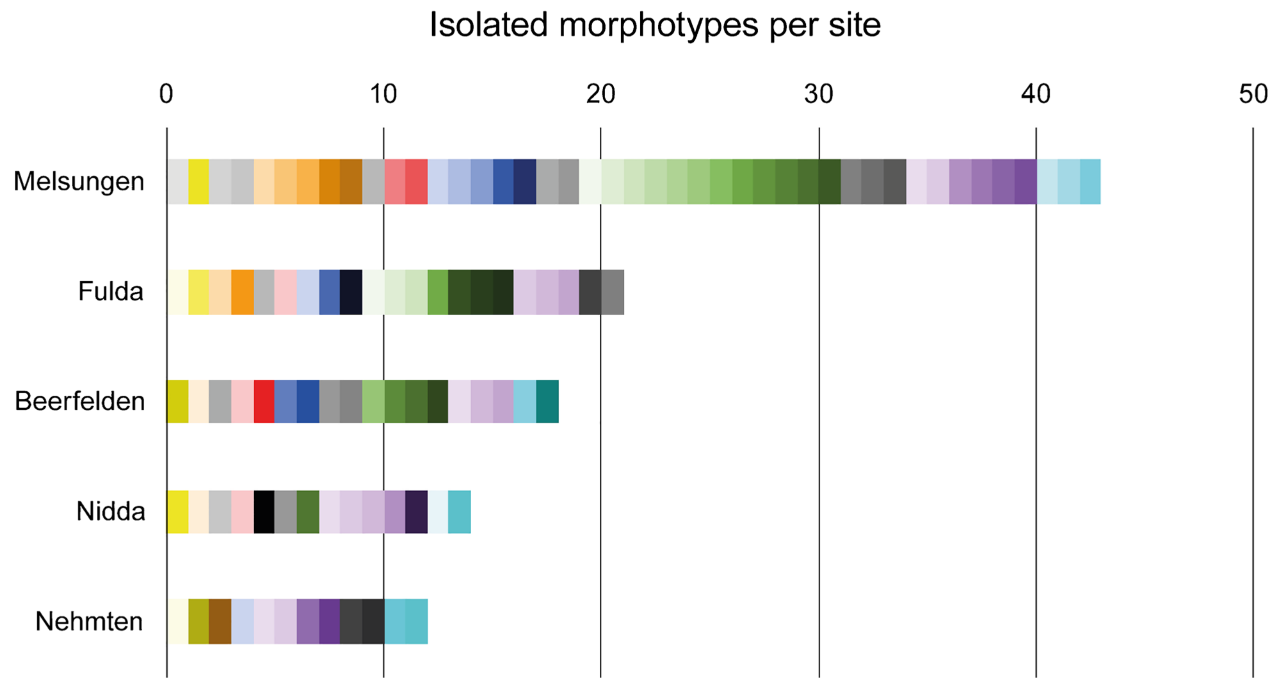

Fig. 2 Overview of isolated morphotypes per site visualized by a stacked bar chart, sorted alphabetically by order within the Ascomycota (*Capnoidales*, yellow; *Diaporthales*, orange; *Helotiales*, red; *Hypocreales*, blue; *Pleosporales*, green; *Xylariales*, purple; remaining orders of the *Ascomycota*, grey, and *Basidiomycota*, turquoise)

Kumm., *Porostereum spadiceum* (Pers.) Hjortstam & Ryvarden, *Serpula himantioides* (Fr.) P. Karst., *Stereum* cf. *hirsutum* (NW-FVA 6270), and *Trametes versicolor* (L.) Lloyd.

Of the 91 isolated morphotypes detected, 41 (45%) were isolated more than once and only six morphotypes were obtained ten or more times. The remaining 50 morphotypes (55%) were only isolated once. Between 0 and 19 different morphotypes were found in the studied woody tissue per tree. On average, 3.3 morphotypes were recorded on each tree. From four trees, no isolations could be made. None of the isolated morphotypes was found at all sites and merely 15 out of the 91 morphotypes were found at more than one site. In total, 84.62% of the isolated

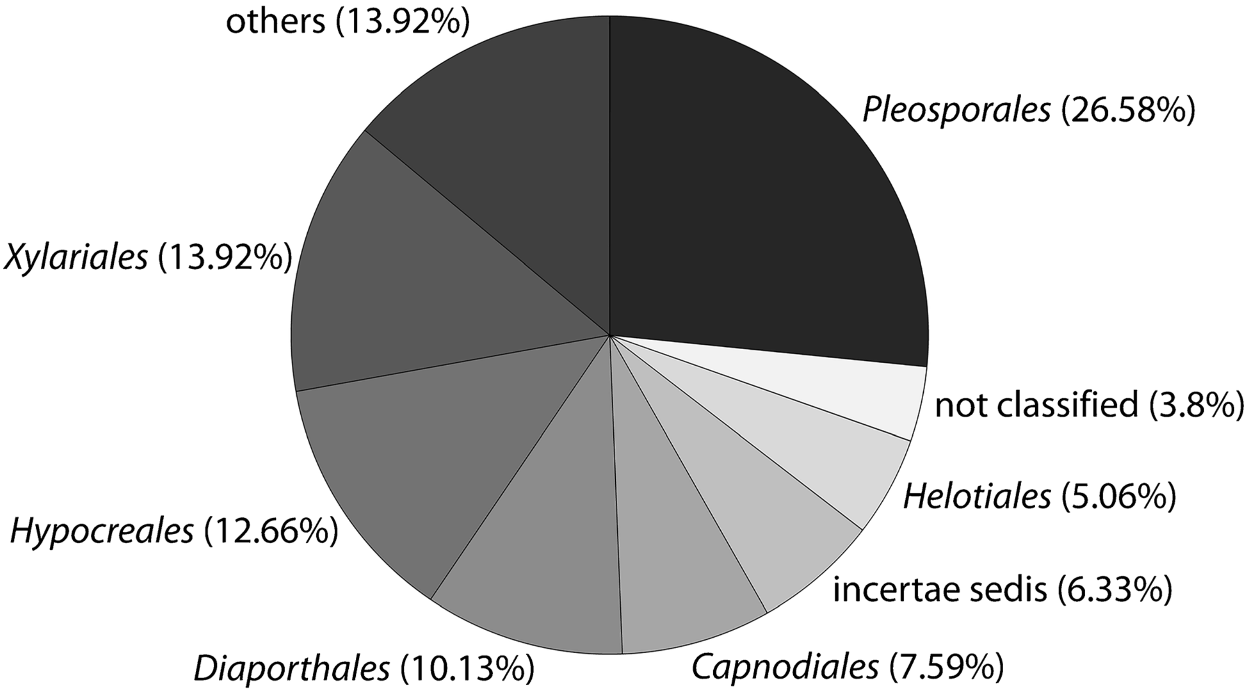

Fig. 3 Isolated orders of the *Ascomycota*, $n = 79$ of the isolated species belonging to the *Ascomycota* (RStudio 4.1.2).

morphotypes were found solely at one site while 63.74% of all isolated morphotypes were isolated from a single tree, respectively. The top ten morphotypes with the highest frequency were *Hymenochaetaceae* sp. (10.6%), *C. corticale* (7.85%), *Cytospora* cf. *populina* (6.48%), *Cytospora* cf. *rodophila* (5.12%), *Thyridium vestitum* (Fr.) Fuckel (4.78%), *Lopadostoma turgidum* (Pers.) Traverso (3.41%), *Xylaria longipes* Nitschke (3.07%), *Penicillium* sp. (3.07%), and *Nectria cinnabarina* (Tode) Fr. (3.07%).

The most abundant morphotypes, in regard to both occurrence at the studied sites and continuity, were *C. corticale* (4 sites, 26% continuity, 7.85% frequency), *X. longipes* (4, 14%, 3.07%), *Lo. turgidum* (3, 10%, 3.41%), *Cadophora prunicola* Damm & S. Bien (3, 6%, 1.02%), *Trichoderma* sp. (3, 6%, 1.02%), *L. muelleri* (2, 12%, 2.05%), *Diaporthe pustulata* Sacc. (2, 6%, 1.02%), *Didymella macrostoma* (Mont.) Qian Chen & L. Cai (2, 6%, 2.05%), and *B. nummularia* (2, 6%, 1.71%).

The number of morphotypes isolated at the different sites ranged from 13 to 44. Hence, the fungal communities at the sites studied differed in their species composition and diversity (Fig. 4). In Melsungen, 44 morphotypes (48.35% of all isolated taxa) were found and 36 (39.56%) of those occurred exclusively at this site. The most common isolated morphotypes in Melsungen, sorted by continuity, were *C. corticale*, *Penicillium* sp., *Cytospora* cf. *rodophila*, *Cytospora* cf. *populina*, and *T. vestitum*. *Cryptostroma corticale* was isolated from 60% of the trees studied at this site. Twenty-one morphotypes (23.07%) were found in the samples from the stand in Fulda, eleven of which (12.01%) were only found there. The most common species at that site were *X. longipes*, *L. muelleri*, *Lo. turgidum*, *D. pustulata*, and *Neonectria* sp. *Cryptostroma corticale* was not isolated from any tree in Fulda. At the Beerfelden study site, 19 morphotypes (20.9%) were found, 13 of which (14.29%) were found exclusively at that site. The five most frequently found species were *Arthrinium rasikravindrae* Shiv M. Singh, L.S. Yadav, P.N. Singh, Rah. Sharma & S.K. Singh, *Arthrinium* cf. *marii*, *L. muelleri*, *C. corticale*, and *Lophiostoma carpini* Andreasen, Jaklitsch & Voglmayr. *Cryptostroma corticale* was found in one of the trees studied (10%). Fifteen morphotypes were found in the Nidda samples (16.5%), eight of which (8.8%) were exclusive to that site, namely *C. corticale*, *B. nummularia*, *Lo. turgidum*, *Hymenochaetaceae* sp., and an unidentified species (NW-FVA 7196). *Cryptostroma corticale* was isolated from

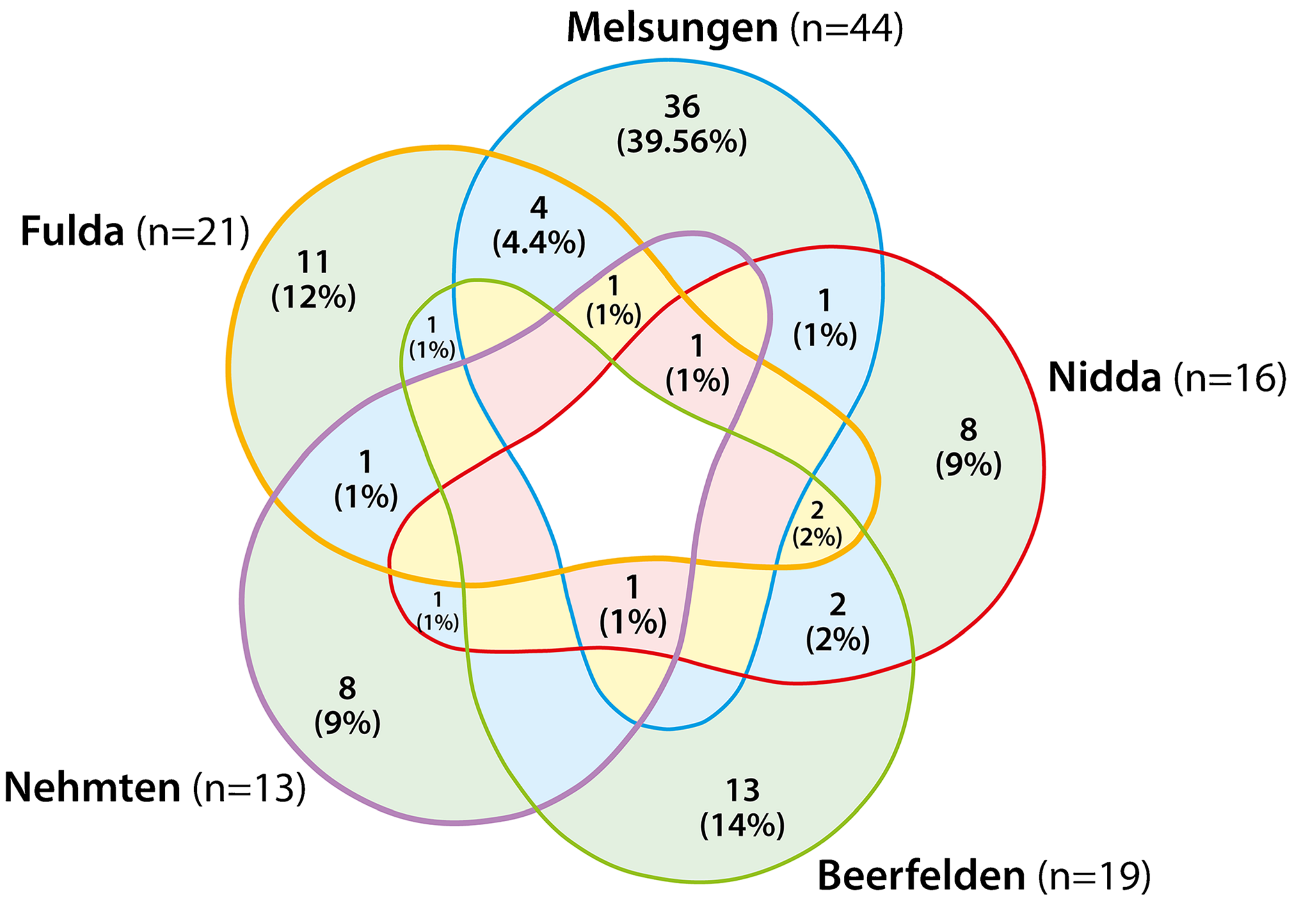

Fig. 4 Overlap between the fungi isolated from each site with the indication of how many of the isolated fungi were found at each site and between the different sites (absolute number as well as percentage); green filling, fungi isolated just at the respective site; blue, fungi isolated from two sites; yellow, fungi isolated at three sites; red, fungi isolated from four sites (RStudio 4.1.2)

five out of ten trees studied in Nidda (50%). At Nehmten, 13 morphotypes (14.29%), including *C. corticale* (10%), were isolated in total, eight of which (8.8%) were found exclusively at that site. At that location, only *Hypoxylon fragiforme* (Pers.) J. Kickx f., was isolated multiple times.

According to the statistical analyses including the distance matrix, *C. corticale* appears to have no significant influence on the fungal community observed in this study. However, eleven fungi only grew out from sampled trees where no *C. corticale* was found (*Agaricales* sp., *Angustimassarina* sp., *A. rasikravindrae*, *Cladosporium* sp. 1, *D.* cf. *rudis*, *D. pustulata*, *L. muelleri*, *Lo. turgidum*, *Neocucurbitaria acerina* Wanas., Camporesi, E.B.G. Jones & K.D. Hyde, *Neodidymelliopsis* sp., and *Neosetophoma* cf. *italica*). Only two fungi (*B. nummularia* and *Capronia* sp.) grew out from trees with *C. corticale*. The third increment taken per tree at Melsungen, increasing the sampling size in contrast to the other sampling sites, had no significant influence on the result of Melsungen being the most diverse site.

Discussion

Composition of isolated fungi

Similar to other studies on fungi isolated from tree woody tissues (Singh et al. 2017; Bußkamp 2018; Ghobad-Nejhad et al. 2018; Langer et al. 2021), in this study mainly *Ascomycota* (85.17%) and significantly less *Basidiomycota* (9.89%) were isolated. This also corresponds to studies focusing on fungi colonising different tree tissues (Petrini and Fisher 1988; Kowalski and Kehr 1992; Peršoh et al. 2010; Martínez-Álvarez et al. 2012; Sanz-Ros et al. 2015). *Basidiomycota* associated with woody tissues are found less frequently in living trees, a reason for this could be that a number of them is involved in wood decay (Langer et al. 2021).

Sieber (2007) concluded that the endophytic fungal communities in *Aceraceae* are mainly dominated by species belonging to *Diaporthales*. Furthermore, *Pleosporales* and *Xylariales* can be dominant endophytes in angiosperms. The morphotypes identified in this study mainly belong to *Pleosporales*, followed by *Xylariales* and *Hypocreales*. Isolates belonging to the *Diaporthales* constitute only the fourth most common group. In comparable studies focusing solely on woody sycamore tissue (e.g. Butin and Kowalski (1986), Kowalski and Kehr (1992), Unterseher et al. (2005), Brglez et al. (2020a)) the most commonly isolated orders include *Diaporthales*, *Helotiales*, *Hypocreales*, and *Pleosporales*. *Diaporthales* did not unequivocally dominate in any of these studies. This discrepancy in the fungal species composition could be explained by the fact that Sieber (2007) focused on different forest trees and fungi isolated from leaves and woody tissue except roots. The composition of fungal orders inhabiting leaves and woody tissue, as subsumed by Sieber (2007), might not represent the composition solely in woody tissue of *Acer* trees in specific. The fungal community of this and the aforementioned studies were not dominated by a few host-specific species as stated by Sieber (2007). In our study, only five species (*C. corticale*, *L. muelleri*, *Lo. turgidum*, *Penicillium* sp., and *X. longipes*) were found in 10% or more of the examined trees. Only one of these is host genus-specific, namely the most abundant, invasive species *C. corticale*. This might be due to the small geographical region that the analysed sites are located in, in comparison to the geographical area covered in the study of Sieber (2007), as well as the diversity of the studied plants or even the differing sampling and isolation methods used.

The total amount of isolated morphotypes from woody sycamore tissue in this study (91) is significantly higher than that in previous investigations, where 10–52 different morphotypes were detected (Butin and Kowalski 1986; Kowalski and Kehr 1992; Unterseher et al. 2005; Brglez et al. 2020a). This difference in diversity can be explained by the larger sampling size and the greater number of sites studied here in contrast to the other studies.

Many of the detected morphotypes in our study were single isolates, which may indicate a sporadic occurrence or sampling bias since only ten trees per site were studied at one specific height. However, it cannot be ruled out that these fungi occur in other stands as well as in higher abundancy and simply were not isolated from the sampled material. Due to the rather small sample size of two or three increments compared to the entire wood body of the tree, the listed fungi are likely just a small fraction of the present fungal community. Additionally, it is to be expected that the composition of fungi might differ within the tree, depending on the host tissue type (Gennaro et al. 2003) and tree age (Halley et al. 1994; Maherali and Klironomos 2007). Furthermore, a possible underestimation of fungal diversity in the studied trees may occur since not all fungi are detectable through standardised culture-based methods or in general (Guo et al. 2001; Allen et al. 2003; Unterseher 2007; Muggia et al. 2017). The composition of the forest stands combined with the nutrient and water availability could also be a factor in assessing the differences in fungal diversity per stand. The stand in Nehmten, stocked only with sycamore, had the lowest fungal diversity of all studied sites and at the same time the lowest nutrient availability.

The composition of fungi isolated in this study differed significantly between the studied forest sites, with very little overlap between the sites. It can be assumed that adding another differing site an entirely new set of fungal wood inhabitants not recorded in this study could be revealed. While some of the isolated fungi (29.1%) were already described as associated with maple (Ellis and Ellis 1985; Butin and Kowalski 1986; Chlebicki 1988; Kowalski and Kehr 1992; Unterseher et al. 2005; Brglez et al. 2020a), most were not recorded in the aforementioned articles (70.9%). Only ten of the detected species (*C. corticale*, *D. pustulata*, *D. rudis*, *D. macrostroma*, *Eutypa maura* (Fr.) Fuckel, *N. cinnabarina*, *N. acerina*, *Petrakia irregularis* Aa, *T. vestitum*, and *X. longipes*) were listed for *A. pseudoplatanus* in the USDA fungal database (Farr and Rossman 2022). Three of them were also reported from sycamore in Germany. This further illustrates the current lack of knowledge about endophytes, specifically in woody tissue of *A. pseudoplatanus* in Germany. According to our data, no difference in the fungal composition per site was observed between trees with *C. corticale* and trees without. These results should be verified by more comprehensive studies on a larger scale.

Function of the isolated fungi

Since our goal was to isolate fungi that were expressing an endophytic life stage in *A. pseudoplatanus* wood, including the check for the presence of *C. corticale*, the far majority of the wood samples we isolated came from trees and tissue that were not exhibiting any SBD symptoms. However, a significant number of fungi have been found to switch between different lifestyles (Promputtha et al. 2010; Álvarez-Loayza et al. 2011; Eaton et al. 2011; O'Connell et al. 2012; Kuo et al. 2015). They live inside their host endophytically, inducing no visible symptoms; however, they become pathogenic, if the host plant is exposed to stress, e.g. drought (Desprez-Loustau et al. 2006; Slippers and Wingfield 2007). Therefore, inferences about the specific function of each species within the temporal-spatial succession of fungal communities linked to healthy wood of *A. pseudoplatanus* trees in Germany must be taken cautiously.

The ten most abundant fungi, including *C. corticale*, were isolated from healthy wood tissue and are discussed hereinafter. It must be assumed that all of them exhibit an endophytic life stage, even though three of them were also isolated from discoloured tissue once as well (*B. nummularia*, *C. corticale*, and *L. muelleri*). This observation for the latter three species is supported by their affiliation to the *Xylariales*, which is an order hosting many wood-decaying fungi with endophytic life stages (Hendry et al. 2002; Bußkamp et al. 2020). *Xylaria longipes* was the second most abundant species in this study and is a known endophyte in a number of different tree species. *Xylaria* species often grow and sporulate on lying deadwood and stumps (Scholtysik et al. 2013). The third most abundant morphotype, assigned to *Lo. turgidum*, belongs to the *Xylariales* as well, and has to the authors' knowledge not been reported from *Acer* before. *Cadophora prunicola*, also belonging to the ten most abundant morphotypes, is only known from its first description on *Prunus* (Bien and Damm 2020a), besides our study. This is the first report of this helotialean species from *A. pseudoplatanus*. For *C. prunicola*, an endophytic life stage is probable, since several other fungi belonging to the *Helotiales* are known endophytes (Petrini and Fisher 1988; Langer et al. 2021). Species of *Trichoderma* can occur as endophytes as described by Evans et al. (2003), while the superordinate order *Hypocreales* is a group hosting several other endophytic species as well. The isolation of *Diaporthe pustulata* in this study was of no surprise since it was originally described from *A. pseudoplatanus* (Gomes et al. 2013). Species belonging to the *Diaporthales* can occur as endophytes (Suryanarayanan 2011; Bußkamp et al. 2020; Langer et al. 2021). Therefore and because it was isolated from healthy tissue, *D. pustulata* might also have an endophytic stage in its life cycle. The pleosporalean fungus *Didymella macrostroma* is presumed to have an endophytic life stage as well, since many other fungi in the *Pleosporales* have endophytic life stages (Langer et al. 2021). *Biscogniauxia nummularia* is a known and widespread multi-host endophyte isolated from several different tree species (Petrini-Klieber 1985; Chapela and Boddy 1988; Chapela 1989; Nugent et al. 2005; Bußkamp et al. 2020) belonging to the *Xylariales* as well, and very common on beech trees (Chapela and Boddy 1988; Chapela 1989). To the authors' knowledge, this fungus has not been reported from *Acer* before.

Occurrence of SBD and spread of *C. corticale*

The observed widespread occurrence of SBD in Germany coincides with information from neighbouring central European countries (Oliveira Longa et al. 2016; Kelnarová et al. 2017; Cech 2019; Queloz et al. 2020). As shown by the SBD reports documented in this study, there are no cases of the disease in the forests of the most northern federal state of Germany, Schleswig-Holstein. This might be due to a more maritime and thus more humid climate. When comparing the site conditions of the forest stand where the fungal wood inhabitants were isolated, it can also be observed that the site in Nehmten, Schleswig-Holstein, has a different bedrock and higher water storing capacity than the other sites (Table 1). Additionally, the site in Nehmten is influenced by groundwater due to being located in a lake region. This indicates that even in drought periods, the plant water availability is more stable, resulting in less plant stress. The climate data for the summers 2018 and 2019 show that temperatures in Hesse were in general higher, as well as in relation to the average reference period with less precipitation in comparison to those in Schleswig-Holstein (DWD 2018, 2019), indicating potential higher drought stress and thus more favourable circumstances for the outbreak of SBD in Hesse, especially with reference to the respective site conditions (Table 1).

The results of Kelnarová et al. (2017) indicate an endophytic life stage of *C. corticale* as it could be detected in part from healthy woody tissue and in a quarter of all studied urban sycamore trees in Prague. However, the study only states that wood discolouration was observed in 59% of all sampled trees and it was not clarified whether *C. corticale* was isolated from discoloured or healthy tissue, only a positive correlation between wood discolouration and the presence of *C. corticale* was mentioned. This assumption is confirmed by our observations of the latent non-symptomatic stage of *C. corticale* in forest trees in Germany. This is the first verification of an endophytic life stage for *C. corticale*. As shown in this study, 26% of all observed, apparently healthy sycamores harbour *C. corti*cale non-symptomatically, regardless of the occurrence of external symptoms in the studied forest stands or area. This leads to the assumption that latent infection with *C. corticale* is widespread in Germany. The risk of mortality due to *C. corticale* for apparently healthy sycamore trees rises with the increase of years with drought and extraordinary high temperatures, since SBD is triggered by these circumstances (Dickenson 1980; Ogris et al. 2021). Even though only vital trees with no signs of distress or injury were chosen for our sampling, 35% of the increments exhibited wood discolouration and/or wood rot. In Kelnarová et al. (2017), discolouration was observed in 59% of all sampled trees and was correlated strongly with the presence of *C. corticale*. In contrast, in this study, *C. corticale* was isolated only once from discoloured woody tissue. Besides *C. corticale*, other wood decay fungi such as *B. nummularia*, *Hymenochaetaceae* sp., and *J. cohaerens* were associated with the discoloured tissues. However, the amount of discoloured tissue observed in this study was rather low, due to our focus on vital trees.

C. corticale was isolated from two of the three stands without obvious symptoms of SBD (Beerfelden, Melsungen, and Nehmten). Especially the proof of *C. corticale* in Nehmten was surprising since symptoms of SBD have not yet been reported from forests in the entire federal state of Schleswig-Holstein. The detection of *C. corticale* in Beerfelden was unexpected, due to the high wood quality and tree vitality of the sampled stand. In contrast, the isolation of *C. corticale* in Nidda was expected, due to obvious symptoms of SBD at the site. The fungus was not detected at the stand of Fulda despite obvious symptoms. Similar to the results of Kelnarová et al. (2017), who used both a culture and a non-culture–based isolation method, the number of trees *C. corticale* was isolated from in our study was higher than expected from the occurrence of SBD with the exception of Fulda. The continuity of *C. corticale* in the presented study is 26%, similar to the results of Kelnarová et al. (2017) where the continuity, based on both isolation methods combined, was 25%.

Conclusion

The results in this paper illustrate once more that *C. corticale* can be isolated from healthy woody tissue of sycamore, thus confirming a latent life stage of this pathogenic fungus. It can be assumed that the current occurrence of the endophytic stage of *C. corticale* associated with *A. pseudoplatanus* is more widely spread than expected. This increases the risk of further outbreak of SBD in forests in the light of climate change. Therefore, the spread of *C. corticale* should be further investigated in Germany as well as in Europe in order to estimate the potential risk for *Acer* trees in this region. The presented data functions as a basis in this endeavour. An increase of negative effects due to climate change, such as rising temperature and insufficient precipitation, needs to be expected for the future, and constitutes major risks for the forest sector. The detected isolated morphotypes could be a basis for future studies concerning potential biological control agents against *C. corticale*.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11557-022-01861-6.

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Author Contribution The study including sampling, lab work, and analysis was primarily conducted by R. Schlößer with support from G. Langer and S. Bien. The first draft of the manuscript was written by R. Schlößer and revised by S. Bien, G. Langer, and E. Langer.

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Data availability The DNA sequences generated in this study were deposited in GenBank (https://www.ncbi.nlm.nih.gov; Table 2). All sampling data is provided in the online resources (ESM 1 and ESM 2).

Declarations

Ethics approval Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

References

Allen TR, Millar T, Berch SM, Berbee ML (2003) Culturing and direct DNA extraction find different fungi from the same ericoid mycorrhizal roots. New Phytol 160:255–272. https://doi.org/10.1046/j.1469-8137.2003.00885.x

Altschul SF, Madden TL, Schäffer AA et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein databases search programs. Nucleic Acids Res 25:3389–3402. https://doi.org/10.1093/nar/25.17.3389

Álvarez-Loayza P, White JF, Torres MS et al (2011) Light converts endosymbiotic fungus to pathogen, influencing seedling survival and niche-space filling of a common tropical tree, *Iriartea deltoidea*. PLoS One 6:e16386. https://doi.org/10.1371/journal.pone.0016386

Anderson IC, Parkin PI (2007) Detection of active soil fungi by RT-PCR amplification of precursor rRNA molecules. J Microbiol Methods 68:248–253. https://doi.org/10.1016/j.mimet.2006.08.005

Andreasen M, Skrede I, Jaklitsch WM et al (2021) Multi-locus phylogenetic analysis of lophiostomatoid fungi motivates a broad concept of *Lophiostoma* and reveals nine new species. Persoonia - Mol Phylogeny Evol Fungi. https://doi.org/10.3767/persoonia.2021.46.09

Arnold AE, Lutzoni F (2007) Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? Ecology 88:541–549

Aveskamp MM, de Gruyter J, Woudenberg JHC et al (2010) Highlights of the *Didymellaceae*: a polyphasic approach to characterise *Phoma* and related pleosporalean genera. Stud Mycol 65:1–60. https://doi.org/10.3114/sim.2010.65.01

Bencheva S (2014) First report of *Cryptostroma corticale* (Ellis & Everh.) P.H. Greg. & S. Waller on *Acer platanoides* L. in Bulgaria. Silva Balcania 15:101–104

Bensch K, Groenewald JZ, Meijer M et al (2018) *Cladosporium* species in indoor environments. Stud Mycol 89:177–301. https://doi.org/10.1016/j.simyco.2018.03.002

Bien S, Damm U (2020a) *Arboricolonus simplex* gen. et sp. nov. and novelties in *Cadophora*, *Minutiella* and *Proliferodiscus* from *Prunus* wood in Germany. MycoKeys 63:119–161. https://doi.org/10.3897/mycokeys.63.46836

Bien S, Damm U (2020b) *Prunus* trees in Germany - a hideout of unknown fungi? Mycol Prog 19:667–690. https://doi.org/10.1007/s11557-020-01586-4

Bien S, Kraus C, Damm U (2020) Novel collophorina-like genera and species from *Prunus* trees and vineyards in Germany. Persoonia - Mol Phylogeny Evol Fungi 45:46–67. https://doi.org/10.3767/persoonia.2020.45.02

Bills GF, Platas G, Peláez F, Masurekar P (1999) Reclassification of a pneumocandin-producing anamorph, *Glarea lozoyensis* gen. et sp. nov., previously identified as *Zalerion arboricola*. Mycol Res 103:179–192. https://doi.org/10.1017/S095375629800687X

Boddy L, Rayner ADM (1983) Origins of decay in living deciduous trees: the role of moisture content and a re-appraisal of the expanded concept of tree decay. New Phytol 94:623–641. https://doi.org/10.1111/j.1469-8137.1983.tb04871.x

Bork K (2018) Rußrindenkrankheit an Ahorn – Erstfund in Bayern. AFZ – Der Wald 20:40–41

Brglez A, Piškur B, Ogris N (2020a) *Eutypella parasitica* and other frequently isolated fungi in wood of dead branches of young sycamore maple (*Acer pseudoplatanus*) in Slovenia. Forests 11:467. https://doi.org/10.3390/f11040467

Brglez A, Piškur B, Ogris N (2020b) In vitro interactions between *Eutypella parasitica* and some frequently isolated fungi from the wood of the dead branches of young sycamore maple (*Acer pseudoplatanus*). Forests 11:1072. https://doi.org/10.3390/f11101072

Bueno E, Martin KR, Raguso RA et al (2020) Response of wild spotted wing *Drosophila* (*Drosophila suzukii*) to microbial volatiles. J Chem Ecol 46:688–698. https://doi.org/10.1007/s10886-019-01139-4

Bußkamp J (2018) Schadenserhebung, Kartierung und Charakterisierung des „Diplodia-Triebsterbens" der Kiefer, insbesondere des endophytischen Vorkommens in den klimasensiblen Räumen und Identifikation von den in Kiefer (*Pinus sylvestris*) vorkommenden Endophyten. Dissertation, Universität Kassel

Bußkamp J, Langer GJ, Langer EJ (2020) *Sphaeropsis sapinea* and fungal endophyte diversity in twigs of Scots pine (*Pinus sylvestris*) in Germany. Mycol Prog 19:985–999. https://doi.org/10.1007/s11557-020-01617-0

Butin H, Kowalski T (1986) Die natürliche Astreinigung und ihre biologischen Voraussetzungen. Eur J For Pathol 16:129–138. https://doi.org/10.1111/j.1439-0329.1986.tb01053.x

Cabral A, Groenewald JZ, Rego C et al (2012a) *Cylindrocarpon* root rot: multi-gene analysis reveals novel species within the *Ilyonectria radicicola* species complex. Mycol Prog 11:655–688. https://doi.org/10.1007/s11557-011-0777-7

Cabral A, Rego C, Nascimento T et al (2012b) Multi-gene analysis and morphology reveal novel *Ilyonectria* species associated with black foot disease of grapevines. Fungal Biol 116:62–80. https://doi.org/10.1016/j.funbio.2011.09.010

Cech TL (2004) Bermerkenserte Pilzkrankheiten in 2004. Forstsch Aktuell 32:31–34

Cech TL (2019) Rußrindenkrankheit bedroht Ahornbestände in Laubwäldern im Osten Niederösterreichs. Forstsch Aktuell 65:24

Chapela IH (1989) Fungi in healthy stems and branches of American beech and aspen: a comparative study. New Phytol 113:65–75. https://doi.org/10.1111/j.1469-8137.1989.tb02396.x

Chapela IH, Boddy L (1988) Fungal colonization of attached beech branches. I. Early stages of development of fungal communities. New Phytol:39–45. https://doi.org/10.1111/j.1469-8137.1988.tb00235.x

Chlebicki A (1988) Some ascomycetous fungi or their anamorphs occurring on trees in Poland I. Acta Mycol 24:77–92

Crous P, Wingfield MJ, Schumacher RK et al (2020) New and interesting fungi. 3. Fungal Syst Evol 6:157–231. https://doi.org/10.3114/fuse.2020.06.09

Crous PW, Schumacher RK, Akulov A et al (2019) New and interesting fungi. 2. Fungal Syst Evol 3:57–134. https://doi.org/10.3114/fuse.2019.03.06

Crous PW, Schumacher RK, Wingfield MJ et al (2015) Fungal systematics and evolution: FUSE 1. Sydowia 67:81–118. https://doi.org/10.12905/0380.sydowia67-2015-0081

Delb H, Grüner J, Reinhold J, et al (2019) Waldschutzsituation 2018/2019 in Rheinland-Pfalz und Saarland. 2019 22–25

Desprez-Loustau M-L, Marçais B, Nageleisen L-M et al (2006) Interactive effects of drought and pathogens in forest trees. Ann For Sci 63:597–612. https://doi.org/10.1051/forest:2006040

Dickenson SJ (1980) Biology of *Cryptostroma corticale* and the sooty bark disease of sycamore. Dissertation, University of Bath

DWD (2018) Deutschlandwetter im Sommer 2018. DWD (Deutscher Wetterdienst), Offenbach

DWD (2019) Deutschlandwetter im Sommer 2019. DWD (Deutscher Wetterdienst), Offenbach

DWD (2020) Deutschlandwetter im Sommer 2020. DWD (Deutscher Wetterdienst), Offenbach

Eaton CJ, Cox MP, Scott B (2011) What triggers grass endophytes to switch from mutualism to pathogenism? Plant Sci 180:190–195. https://doi.org/10.1016/j.plantsci.2010.10.002

Ellis JB, Everhart BM (1889) New species of hyphomycetes fungi. J Mycol 5:68–72

Ellis MB, Ellis PJ (1985) Microfungi on land plants: an identification handbook. Croom Helm Ltd., London

Enderle R, Riebesehl J, Becker P, Kehr R (2020) Rußrindenkrankheit an Ahorn - Biologie, Pathologie und Entsorgung von Schadholz. In: Jahrbuch der Baumpflege 2020, 24th edn. Haymarket Media, Braunschweig, pp 85–100

Engesser R, Forster B, Meier E, Odermatt O (2004) Forstschutzsituation 2003 in der Schweiz. AFZ – Der Wald 59:385–387

EUFORGEN (2022) *Acer pseudoplatanus* - sycamore. In: Euforgen. http://www.euforgen.org/species/acer-pseudoplatanus/. Accessed 25 Mar 2022

Evans HC, Holmes KA, Thomas SE (2003) Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and a preliminary assessment of their potential as biocontrol agents of cocoa diseases. Mycol Prog 2:149–160. https://doi.org/10.1007/s11557-006-0053-4

Farr DF, Rossman AY (2022) Fungal databases - fungus-host distributions. In: US Natl. Fungus Collect. Fungal database. https://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm. Accessed 4 May 2022

Frisvad JC, Houbraken J, Popma S, Samson RA (2013) Two new *Penicillium* species *Penicillium buchwaldii* and *Penicillium spathulatum* , producing the anticancer compound asperphenamate. FEMS Microbiol Lett 339:77–92. https://doi.org/10.1111/1574-6968.12054

Gao C-H (2021) ggVennDiagram: A “ggplot2” implement of Venn Diagram. In: GgVennDiagram Ggplot2 implement Venn Diagr. https://CRAN.R-project.org/package=ggVennDiagram

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Gennaro M, Gonthier P, Nicolotti G (2003) Fungal endophytic communities in healthy and declining *Quercus robur* L. and *Q. cerris* L. trees in northern Italy. J Phytopathol 151:529–534. https://doi.org/10.1046/j.1439-0434.2003.00763.x

Ghobad-Nejhad M, Meyn R, Langer E (2018) Endophytic fungi isolated from healthy and declining Persian oak (*Quercus brantii*) in western Iran. Nova Hedwigia 107:273–290. https://doi.org/10.1127/nova_hedwigia/2018/0470

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Glynou K, Thines M, Maciá-Vicente JG (2018) Host species identity in annual *Brassicaceae* has a limited effect on the assembly of root-endophytic fungal communities. Plant Ecol Divers 11:569–580. https://doi.org/10.1080/17550874.2018.1504332

Gomes RR, Glienke C, Videira SIR et al (2013) *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia Mol Phylogeny Evol Fungi 31:1

Gregory PH, Waller S (1951) *Cryptostroma corticale* and sooty bark disease of sycamore (*Acer pseudoplatanus*). Trans Br Mycol Soc 34:579–597. https://doi.org/10.1016/S0007-1536(51)80043-3

Guo LD, Hyde KD, Liew ECY (2000) Identification of endophytic fungi from *Livistona chinensis* based on morphology and rDNA sequences. New Phytol 147:617–630

Guo LD, Hyde KD, Liew ECY (2001) Detection and taxonomic placement of endophytic fungi within frond tissues of *Livistona chinensis* based on rDNA sequences. Mol Phylogenet Evol 20:1–13. https://doi.org/10.1006/mpev.2001.0942

Haenzi M, Cochard B, Chablais R et al (2021) *Neofusicoccum parvum*, a new agent of sequoia canker and dieback identified in Geneva, Switzerland. Forests 12:434. https://doi.org/10.3390/f12040434

Hall T (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98. https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29

Halley JM, Comins HN, Lawton JH, Hassell MP (1994) Competition, succession and pattern in fungal communities: towards a cellular automaton model. Oikos 70:435–442. https://doi.org/10.2307/3545783

Hendry SJ, Boddy L, Lonsdale D (2002) Abiotic variables effect differential expression of latent infections in beech (*Fagus sylvatica*). New Phytol 155:449–460. https://doi.org/10.1046/j.1469-8137.2002.00473.x

Hosoya T, Hosaka K, Nam KO (2018) A check list of non-lichenised fungi occurring on *Fagus crenata*, a tree endemic to Japan. Mycology 9:29–34. https://doi.org/10.1080/21501203.2017.1363092

Hyde KD, Soytong K (2008) The fungal endophyte dilemma. Fungal Divers 33:163–173

Itagaki H, Hosoya T (2021) Lifecycle of *Pyrenopeziza protrusa* (*Helotiales*, *Dermateaceae* sensu lato) in *Magnolia obovata* revealed by field observation and molecular quantification. Mycoscience 62:373–381. https://doi.org/10.47371/mycosci.2021.08.001

Izumitsu K, Hatoh K, Sumita T et al (2012) Rapid and simple preparation of mushroom DNA directly from colonies and fruiting bodies for PCR. Mycoscience 53:396–401

Jaklitsch WM, Fournier J, Rogers JD, Voglmayr H (2014) Phylogenetic and taxonomic revision of *Lopadostoma*. Persoonia - Mol Phylogeny Evol Fungi 32:52–82. https://doi.org/10.3767/003158514X679272

Jaklitsch WM, Voglmayr H (2016) Hidden diversity in *Thyridaria* and a new circumscription of the *Thyridariaceae*. Stud Mycol 85:35–64. https://doi.org/10.1016/j.simyco.2016.09.002

Jankowiak R (2005) Fungi associated with *Ips typographus* on *Picea abies* in southern Poland and their succession into the phloem and sapwood of beetle-infested trees and logs. For Pathol 35:37–55. https://doi.org/10.1111/j.1439-0329.2004.00395.x

Jankowiak R, Bilański P, Paluch J, Kołodziej Z (2016) Fungi associated with dieback of *Abies alba* seedlings in naturally regenerating forest ecosystems. Fungal Ecol 24:61–69. https://doi.org/10.1016/j.funeco.2016.08.013

Kelnarová I, Černý K, Zahradník D, Koukol O (2017) Widespread latent infection of *Cryptostroma corticale* in asymptomatic *Acer pseudoplatanus* as a risk for urban plantations. For Pathol 47:e12344. https://doi.org/10.1111/efp.12344

Koukol O, Kelnarová I, Černý K (2014) Recent observations of sooty bark disease of sycamore maple in Prague (Czech Republic) and the phylogenetic placement of *Cryptostroma corticale*. For Pathol 45:21–27. https://doi.org/10.1111/efp.12129

Kowalski T, Bilański P (2021) Fungi detected in the previous year’s leaf petioles of *Fraxinus excelsior* and their antagonistic potential against *Hymenoscyphus fraxineus*. Forests 12:1412. https://doi.org/10.3390/f12101412

Kowalski T, Kehr RD (1992) Endophytic fungal colonization of branch bases in several forest tree species. Sydowia 44:137–168

Kruys Å, Castlebury LA (2012) Molecular phylogeny of *Sydowiellaceae*—resolving the position of *Cainiella*. Mycologia 104:419–426. https://doi.org/10.3852/11-163

Kuhnert E, Fournier J, Peršoh D et al (2014) New *Hypoxylon* species from Martinique and new evidence on the molecular phylogeny of *Hypoxylon* based on ITS rDNA and β-tubulin data. Fungal Divers 64:181–203. https://doi.org/10.1007/s13225-013-0264-3

Kuo H-C, Hui S, Choi J et al (2015) Secret lifestyles of *Neurospora crassa*. Sci Rep 4:5135. https://doi.org/10.1038/srep05135

Langer GJ (1994) Die Gattung *Botryobasidium* DONK (*Corticiaceae*, *Basidiomycetes*). J. Cramer, Berlin, Stuttgart

Langer GJ (2017) Collar rots in forests of Northwest Germany affected by ash dieback. Balt For 23:4–19

Langer GJ, Bressem U, Habermann M (2013) Vermehrt Pilzkrankheiten an Bergahorn in Nordwestdeutschland. AFZ - Der Wald 6:22–26

Langer GJ, Bußkamp J, Blumenstein K, Terhonen E (2021) Fungi inhabiting woody tree tissues – stems, branches, and twigs. In: Forest microbiome. Elsevier, London, San Diego, Cambridge, Oxford, pp 175–205

Li WJ, McKenzie EHC, Liu JK et al (2020) Taxonomy and phylogeny of hyaline-spored coelomycetes. Fungal Divers 100:279–801. https://doi.org/10.1007/s13225-020-00440-y

Lygis V, Vasiliauskas R, Larsson K-H, Stenlid J (2005) Wood-inhabiting fungi in stems of *Fraxinus excelsior* in declining ash stands of northern Lithuania, with particular reference to *Armillaria cepistipes*. Scand J For Res 20:337–346

Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316:1746–1748. https://doi.org/10.1126/science.1143082

Martínez-Álvarez P, Rodríguez-Ceinós S, Martín-García J, Diez JJ (2012) Monitoring endophyte populations in pine plantations and native oak forests in Northern Spain. For Syst 21:373–382. https://doi.org/10.5424/fs/2012213-02254

Metzler B (2006) *Cryptostroma corticale* an Bergahorn nach dem Trockenjahr 2003. Mitteilungen Biol Bundesanst Für Land-Forstwirtsch 400:161–162

Muggia L, Kopun T, Grube M (2017) Effects of growth media on the diversity of culturable fungi from lichens. Molecules 22:824. https://doi.org/10.3390/molecules22050824

Nugent LK, Sihanonth P, Thienhirun S, Whalley AJS (2005) *Biscogniauxia*: a genus of latent invaders. Mycologist 19:40–43. https://doi.org/10.1017/S0269915X05001060

NW-FVA (2020) Waldschutzinfo Nr. 1 / 2020 - Witterung 2019. Nordwestdeutsche Forstliche Versuchsanstalt, Göttingen

NW-FVA (2021) Waldschutzinfo Nr. 1 / 2021 Witterung 2020. Nordweestdeutsche Forstliche Versuchsanstalt, Göttingen

O'Connell RJ, Thon MR, Hacquard S et al (2012) Lifestyle transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome and transcriptome analyses. Nat Genet 44:1060–1065. https://doi.org/10.1038/ng.2372

O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol Phylogenet Evol 7:103–116. https://doi.org/10.1006/mpev.1996.0376

Ogris N, Brglez A, Piškur B (2021) Drought stress can induce the pathogenicity of *Cryptostroma corticale*, the causal agent of Sooty Bark Disease of sycamore maple. Forests 12:377. https://doi.org/10.3390/f12030377

Oksanen J, Simpson GL, Guillaume Blanchet F, et al (2022) vegan: community ecology package. In: Vegan community ecol. package. https://CRAN.R-project.org/package=vegan. Accessed 20 Sep 2022

Oliveira Longa CM, Vai N, Maresi G (2016) *Cryptostroma corticale* in the northern Apennines (Italy). Phytopathol Mediterr 55:136–138. https://doi.org/10.14601/Phytopathol_Mediterr-17164

Parfitt D, Hunt J, Dockrell D et al (2010) Do all trees carry the seeds of their own destruction? PCR reveals numerous wood decay fungi latently present in sapwood of a wide range of angiosperm trees. Fungal Ecol 3:338–346. https://doi.org/10.1016/j.funeco.2010.02.001

Peršoh D, Melcher M, Flessa F, Rambold G (2010) First fungal community analyses of endophytic ascomycetes associated with *Viscum album* ssp. *austriacum* and its host *Pinus sylvestris*. Fungal Biol 114:585–596. https://doi.org/10.1016/j.funbio.2010.04.009

Petrini O (1991) Fungal endophytes of tree leaves. In: Andrews HJ, Hirano SS (eds) Microbial ecology of leaves. Springer-Verlag, New York and Berlin, pp 179–197

Petrini O, Fisher PJ (1988) A comparative study of fungal endophytes in xylem and whole stem of *Pinus sylvestris* and *Fagus sylvatica*. Trans Br Mycol Soc 91:233–238. https://doi.org/10.1016/S0007-1536(88)80210-9

Petrini-Klieber LE (1985) Untersuchungen über die Gattung *Hypoxylon* (*Ascomycetes*) und verwandte Pilze. Dissertation, ETH Zürich

Plate HP, Schneider R (1965) Ein Fall von asthmaartiger Allergie, verursacht durch den Pilz *Cryptostroma corticale*. Nachrichtenblatt Dtsch Pflanzenschutzdienstes 17:100–101

Promputtha I, Hyde KD, McKenzie EHC et al (2010) Can leaf degrading enzymes provide evidence that endophytic fungi becoming saprobes? Fungal Divers 41:89–99. https://doi.org/10.1007/s13225-010-0024-6

Quaedvlieg W, Verkley GJM, Shin HD et al (2013) Sizing up *Septoria*. Stud Mycol 75:307–390

Queloz V, Forster B, Beenken L, et al (2020) Waldschutzüberblick 2019. Eidg. Forschungsanstalt für Wald, Schnee und Landschaft WSL, CH-8903 Birmensdorf

R Core Team (2021) R: a language and environment for statistical computing.
R Foundation for Statistical Computing

Ragazzi A, Moricca S, Capretti P et al (2003) Differences in composition of endophytic mycobiota in twigs and leaves of healthy and declining *Quercus* species in Italy. For Pathol 33:31–38. https://doi.org/10.1046/j.1439-0329.2003.3062003.x

Robeck P, Heinrich R, Schumacher J et al (2008) Status der Rußrindenkrankheit des Ahorns in Deutschland. In: Jahrbuch der Baumpflege. Haymarket Media GmbH, Braunschweig, pp 238–244

Rohde M, Langer G, Hurling R, Plašil P (2019) Waldschutzsituation 2018 in Nordwestdeutschland. AFZ – Der Wald 74:38–41

Runge F (1994) Die Pflanzengesellschaften Mitteleuropas, 12/13 edn. Aschendorff Verlag, Münster (Westfalen)

Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998) Fungal endophytes: a continuum of interactions with host plants. Annu Rev Ecol Syst 29:319–343

Saikkonen K, Saari S, Helander M (2010) Defensive mutualism between plants and endophytic fungi? Fungal Divers 41:101–113. https://doi.org/10.1007/s13225-010-0023-7

Samuels GJ, Dodd SL, Lu BS et al (2006) The *Trichoderma koningii* aggregate species. Stud Mycol 56:67–133. https://doi.org/10.3114/sim.2006.56.03

Sanz-Ros AV, Müller MM, San Martín R, Diez JJ (2015) Fungal endophytic communities on twigs of fast and slow growing Scots pine (*Pinus sylvestris* L.) in northern Spain. Fungal Biol 119:870–883. https://doi.org/10.1016/j.funbio.2015.06.008

Schlegel M, Queloz V, Sieber TN (2018) The endophytic mycobiome of European ash and sycamore maple leaves – geographic patterns, host specificity and influence of ash dieback. Front Microbiol 9:2345. https://doi.org/10.3389/fmicb.2018.02345

Schlößer R, Langer G (2021) Verbreitung der Rußrindenkrankheit des Ahorns in Deutschland. AFZ – Der Wald 24:28–32

Schmidt PA (2009) Der Bergahorn – eine typische Mischwald-Baumart süd-mitteleuropäischer Bergwälder. In: Bayerische Landesanstalt für Wald und Forstwirtschaft (LWF) (ed) Beiträge zum Bergahorn. Freising, pp 13–18

Scholtysik A, Unterseher M, Otto P, Wirth C (2013) Spatio-temporal dynamics of endophyte diversity in the canopy of European ash (*Fraxinus excelsior*). Mycol Prog 12:291–304. https://doi.org/10.1007/s11557-012-0835-9

Schulthess FM, Faeth SH (1998) Distribution, abundances, and associations of the endophytic fungal community of Arizona fescue (*Festuca arizonica*). Mycologia 90:569–578. https://doi.org/10.1080/00275514.1998.12026945

Schwarze FWMR (2018) Diagnose und Prognose der Fäuledynamik in Stadtbäumen. MycoSolutions AG, ST. Gallen

Senanayake I, Al-Sadi AM, Bhat JD et al (2016) *Phomatosporales* ord. nov. and *Phomatosporaceae* fam. nov., to accommodate *Lanspora*, *Phomatospora* and *Tenuimurus*, gen. nov. Mycosphere 7:628–641. https://doi.org/10.5943/mycosphere/7/5/8

Sieber TN (2007) Endophytic fungi in forest trees: are they mutualists? Fungal Biol Rev 21:75–89. https://doi.org/10.1016/j.fbr.2007.05.004

Singh DK, Sharma VK, Kumar J et al (2017) Diversity of endophytic mycobiota of tropical tree *Tectona grandis* Linn.f.: spatiotemporal and tissue type effects. Sci Rep 7:3745. https://doi.org/10.1038/s41598-017-03933-0

Slippers B, Wingfield MJ (2007) *Botryosphaeriaceae* as endophytes and latent pathogens of woody plants: diversity, ecology and impact. Fungal Biol Rev 21:90–106. https://doi.org/10.1016/j.fbr.2007.06.002

Spies CFJ, Mostert L, Carlucci A et al (2020) Dieback and decline pathogens of olive trees in South Africa. Persoonia - Mol Phylogeny Evol Fungi 45:196–220. https://doi.org/10.3767/persoonia.2020.45.08

Sun X, Guo LD, Hyde KD (2011) Community composition of endophytic fungi in *Acer truncatum* and their role in decomposition. Fungal Divers 47:85–95. https://doi.org/10.1007/s13225-010-0086-5

Suryanarayanan TS (2011) Diversity of fungal endophytes in tropical trees. In: Pirttilä AM, Frank AC (eds) Endophytes of forest trees. Springer Netherlands, Dordrecht, pp 67–80

Townrow JA (1953) The Biology of *Cryptostroma corticale* and the sooty bark disease of sycamore. Report on forest research, London, pp 118–120

Travadon R, Lecomte P, Diarra P, et al (2015) Influence of pruning systems on trunk pathogens and other fungi colonizing grapevine wood. Am Phytopathol Soc Abstr 68-O

Tropf J (2020) Mykologische und histologische Untersuchungen im Zusammenhang mit der Rußrindenkrankheit, verursacht durch Cryptostroma corticale. Master's Thesis, Albert-Ludwigs-Universität Freiburg

Trouillas FP, Gubler WD (2004) Identification and characterization of *Eutypa leptoplaca*, a new pathogen of grapevine in Northern California. Mycol Res 108:1195–1204. https://doi.org/10.1017/S0953756204000863

Untereiner WA, Naveau FA (1999) Molecular systematics of the *Herpotrichiellaceae* with an assessment of the phylogenetic positions of *Exophiala dermatitidis* and *Phialophora americana*. Mycologia 91: 67–83. https://doi.org/10.1080/00275514.1999.12060994

Unterseher M (2007) Fungal biodiversity research – from cultivation of foliar tree endophytes to the combination of ecological theory and next generation sequencing. Ernst Moritz Arndt Universität Greifswald, Dissertation

Unterseher M, Otto P, Morawetz W (2005) Species richness and substrate specificity of lignicolous fungi in the canopy of a temperate, mixed deciduous forest. Mycol Prog 4:117–132. https://doi.org/10.1007/s11557-006-0115-7

Utermann C, Echelmeyer VA, Oppong-Danquah E et al (2020) Diversity, bioactivity profiling and untargeted metabolomics of the cultivable gut microbiota of *Ciona intestinalis*. Mar Drugs 19:6. https://doi.org/10.3390/md19010006

Větrovský T, Kolařík M, Žifčáková L et al (2016) The *rpb2* gene represents a viable alternative molecular marker for the analysis of environmental fungal communities. Mol Ecol Resour 16:388–401. https://doi.org/10.1111/1755-0998.12456

Videira SIR, Groenewald JZ, Braun U et al (2016) All that glitters is not *Ramularia*. Stud Mycol 83:49–163. https://doi.org/10.1016/j.simyco.2016.06.001

Voglmayr H, Aguirre-Hudson MB, Wagner HG et al (2019) Lichens or endophytes? The enigmatic genus *Leptosillia* in the *Leptosilliaceae* fam. nov. (*Xylariales*), and *Furfurella* gen. nov. (*Delonicicolaceae*). Persoonia - Mol Phylogeny Evol Fungi 42:228–260. https://doi.org/10.3767/persoonia.2019.42.09

Vu GM, de Vries M et al (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Stud Mycol 92:135–154. https://doi.org/10.1016/j.simyco.2018.05.001

Wanasinghe D, Phookamsak R, Jeewon R et al (2017) A family level rDNA based phylogeny of *Cucurbitariaceae* and *Fenestellaceae* with descriptions of new *Fenestella* species and *Neocucurbitaria* gen. nov. Mycosphere 8:397–414. https://doi.org/10.5943/mycosphere/8/4/2

Wenzel A, Thiel J, Stürtz M (2019) Waldschutzsituation 2018/19 in Thüringen. AFZ - Der Wald 74:26–29

White TJ, Bruns T, Lee SJWT, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc Guide Methods Appl 18:315–322

Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer, New York

Wickham H (2011) The split-apply-combine strategy for data analysis. J Stat Softw 40:1–29

Wickham H, Averick M, Bryan J et al (2019) Welcome to the Tidyverse. J Open Source Softw 4:1686. https://doi.org/10.21105/joss.01686

Worrall JJ, Anagnost SE, Zabel RA (1997) Comparison of wood decay among diverse lignicolous fungi. Mycologia 89:199–219

Wysoczański W, Węgrzyn E, Lembicz M, Jaroszewicz B (2021) Fungal microbiota in seeds, seedlings and mature plants of raspberry (*Rubus ideaus* L.). Eur J Plant Pathol 161:815–820. https://doi.org/10.1007/s10658-021-02364-y

Zhang W, Groenewald JZ, Lombard L et al (2021) Evaluating species in *Botryosphaeriales*. Persoonia - Mol Phylogeny Evol Fungi 46:63–115. https://doi.org/10.3767/persoonia.2021.46.03

Zhao P, Luo J, Zhuang W et al (2011) DNA barcoding of the fungal genus *Neonectria* and the discovery of two new species. Sci China Life Sci 54:664–674. https://doi.org/10.1007/s11427-011-4184-8

Zhou LW, Vlasak JJ, Vlasak J (2014) *Inonotus andersonii* and *I. krawtzewii*: another case of molecular sequencing-based diagnosis of morphologically similar species. Chiang Mai J Sci 41:789–797

3 Manuscript II

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On the lookout for a potential antagonist against *Cryptostroma corticale*—an insight through *in vitro* dual culture studies

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In an effort to find a potential antagonist for *Cryptostroma corticale*, the causal agent of the sooty bark disease, different fungi previously isolated from *Acer pseudoplatanus* were tested in dual culture antagonism assays with *C. corticale*. In total 102 fungal strains, mainly *Ascomycota*, were tested. Each potentially antagonistic strain was paired with three different strains of *C. corticale*. Four different tests were conducted to get a better understanding of the interactions between *C. corticale* and the potential antagonists. Test 1 was a dual culture set up at room temperature with all 102 strains to get an overview of the interactions with *C. corticale*, as well as placing the potential antagonist on a Petri dish 1 week prior to *C. corticale* for a selection of fungi. For Test 2, only fungi which showed inhibition at distance in Test 1 were chosen and tested on different media at 25°C, the optimal growing temperature of the pathogen. In Test 3, fungi showing signs of antagonism in Test 1 were tested against *C. corticale* in a 6:1 ratio of potential antagonist plugs to the *C. corticale* plug. For Test 4, the viability of *C. corticale* hyphae was tested after they stopped growing in dual culture with a potential antagonist. These *in vitro* trials show that *C. corticale* displays a very competitive behavior, overgrowing most of the tested fungi. However, nine of the tested fungal strains, showed an antagonism at distance for at least one of the *C. corticale* strains. Over all tests, *Hypholoma fasciculare*, *Jackrogersella cohaerens*, *Paracamarosporium* cf. *fagi*, *Pezicula sporulosa,* and *Preussia* cf. *aemulans* showed the highest potential in regard of functionality as a biological control agent.

KEYWORDS

fungal endophytes, sooty bark disease, sycamore maple, antagonism, biocontrol

1 Introduction

Cryptostroma corticale (Ellis & Everh.) P. H. Greg. & H. Waller is the causal agent of the sooty bark disease (SBD) in *Acer* trees (Ellis and Everhart, 1889; Gregory and Waller, 1951). It was originally described in 1889 by Ellis and Everhart (1889) as *Coniosporium corticale* from Canada, where it is considered native. It was first detected in Europe in 1945 in Wanstead Park in London, Great Britain by Gregory and Waller (1951) and was also detected around the same time in Paris, France by Moreau and Moreau (1951). The first detection of the disease in Germany dates back to 1964, when it was found on stored wood in a basement in Berlin (Plate and Schneider, 1965). Due to exceptionally warm and dry summers in central Europe in the recent past (Bastos et al., 2020; Schuldt et al., 2020; Hänsel et al., 2022), the SBD has become even more prominent. The disease is associated with wilt symptoms, yellowing of the leaves and wood discoloration (Gregory and Waller, 1951; Dickenson, 1980), which devalues timber of *Acer* trees. The spores produced by the pathogen can cause extrinsic allergic alveolitis or hypersensitivity pneumonitis in humans (Towey et al., 1932; Braun et al., 2021), though there have been no recent reports from Europe. The SBD is present in several European countries such as the Czech Republic, France, Germany, Italy, Switzerland, and the United Kingdom (Gregory and Waller, 1951; Oliveira Longa et al., 2016; Kelnarová et al., 2017; Muller et al., 2023). Some disease outbreaks in Germany have led to the felling of entire severely damaged stands.

Cryptostroma corticale is considered to be an opportunistic pathogen and has been described as a saprotroph (Young, 1978). It was assumed to have an endophytic life stage (Sieber, 2007), becoming pathogenic only under favorable conditions for the pathogen (Enderle et al., 2020), for example heat and drought (Dickenson, 1980; Dickenson and Wheeler, 1981; Eastburn et al., 2011; Enderle et al., 2020). The response of plants to drought and temperature stress may influence other stress responses of plants, including those toward pathogens (Eastburn et al., 2011), and in turn be beneficial for emerging pathogens (Desprez-Loustau et al., 2007), such as *C. corticale*. The optimal growing temperature for *C. corticale* is 25°C (Dickenson, 1980), making rising summer temperatures increasingly suitable for the pathogen. The endophytic stage of *C. corticale* has recently been demonstrated by Schlößer et al. (2023). The systematic placement of *C. corticale* is still being discussed. Molecular analysis showed that *C. corticale* belongs to the *Graphostromataceae* M.E. Barr, J.D. Rogers & Y.M. Ju, Xylariales Tul. & C. Tul. (Koukol et al., 2014). According to the study of Koukol et al. (2014), its closest relatives are *Biscogniauxia bartholomaei* (Peck) Lar. N. Vassiljeva and *Graphostroma platystomum* (Schwein.) Piroz.

There are currently no means available to control *C. corticale* in the living host tree. Fungal antagonists capable of colonizing the same habitat as *C. corticale* present a promising option for biological control due to their endophytic life stage. The use of beneficial living organisms for pest control is broadly defined as biological control (Kenis et al., 2019). Biological control agents (BCA) can promote positive reactions in the plant and reduce negative effects caused by plant pathogens (Shoresh et al., 2010). Species of the genus *Trichoderma* have been recognized for their potential as BCA against plant pathogens since the early 1930s (Howell, 2003). BCA ideally should be organisms that do not cause harm to the target plant, such as fungi in their endophytic stage. Endophytes can modify the severity and expression of host plant diseases through their interaction with pathogens (Busby et al., 2016). Through these interactions endophytes are able to antagonize pathogens via different mechanisms like competition, hyperparasitism or antibiosis (Busby et al., 2016), and can thus function as biological control. BCA are primarily researched in agriculture (Busby et al., 2016) due to this field's economic importance as well as the one-year life-cycle of crops as opposed to the life span of a forest tree (Pratt et al., 1999). A commonly used biocontrol method in agriculture is treating seeds with an antagonist of a problematic pathogen to prevent the initial establishment of the pathogen in the target plant, as described for treatment of tomato seeds with *Trichoderma harzianum* Rifai and *T. koningii* Oudem. strains for protection against *Pythium* sp. (Harman and Taylor, 1988; Taylor and Harman, 1990; Harman, 1991). There are only a few examples from forestry, such as *Phlebiopsis gigantea* (Fr.) Jülich against *Heterobasidion annosum* s.l. (Fr.) Bref., which causes root rot (Rishbeth, 1963; Greig, 1976), and *Cryphonectria hypovirus* 1 (CHV1), which is effective against *Cryphonectria parasitica* (Murr.) Barr causing cankers on *Castanea* spp. (Heiniger and Rigling, 1994; Rigling and Prospero, 2018).

A number of different techniques and methods have been used to study fungal antagonisms, like observing interactions between hyphae and clustering the interactions as types (Yuen et al., 1999). In order to identify such interactions, *in vitro* experiments have to be conducted to investigate the specific interaction between two different fungi. Different interactions between fungi *in vitro* have previously been described by Boddy (2000), namely deadlock and replacement. Deadlock describes a hyphal interaction where both fungi stop their growth, whereas replacement happens when one fungal culture overgrows, i.e., replaces, the other (Boddy, 2000).

The aim of this study was to gain insights into the *in vitro* interaction between the non-native invasive species *C. corticale* and other fungi occurring naturally in sycamore maple (*Acer pseudoplatanus* L.) in Germany. The objective was to identify a fungus which might be a candidate to be used as a BCA against *C. corticale*. For this, four dual culture tests between *C. corticale* and different potentially antagonistic fungal strains isolated from sycamore were conducted: Test 1 to get an overview of the fungal interactions, Test 2 to see if different types of media and the optimal growing temperature of the pathogen have an influence on the interactions, Test 3 to investigate whether *C. corticale* can compete in a growth challenge against six agar plugs colonized by the potentially antagonistic fungus, and Test 4 in order to test viability of hyphal tips of *C. corticale* after they stopped growing in an interaction.

2 Materials and methods

In order to determine the interaction between *C. corticale* strains and the fungi isolated from sycamore maple (hereafter "potential antagonists") by Schlößer et al. (2023), four tests were carried out (Figure 1). Three different strains of *C. corticale* (CC1: NW-FVA 5889, CC2: NW-FVA 7011 and CC3: NW-FVA 7148; Table 1) with different geographical origins and slight differences in the habitus of their pure cultures were chosen for the experiment and used in all four tests. The three *C. corticale* strains behaved very similar in pure culture in terms of growth rate. The 76 potential antagonistic taxa (102 strains) used in this study (Table 2) were obtained from wood core borings and mainly belong to the Ascomycota (88%) and to a lesser extent to the Basidiomycota (11%), one Mucoromycota and one tested fungus could not be taxonomically placed (*Fungus* sp.). Sampling, isolation, and storage of fungal cultures is described in Schlößer et al. (2023). The types of media used in this study were malt yeast peptone agar (MYP), modified according to Langer (1994), and potato dextrose agar (PDA), following the instructions of the manufacturer (Fluka). All 90 mm Petri dishes, were filled with 20 mL of the respective nutrient media.

2.1 Test 1—Dual cultures on MYP

For the dual cultures of Test 1, 76 taxa with 102 strains were tested. For this test the method of Rigerte et al. (2019) was followed regarding set up and measurements (see Figure 2A). For each strain of *C. corticale* (CC1-CC3) triplicates per tested strain were set up. The inoculated Petri dishes were kept at room temperature and ambient

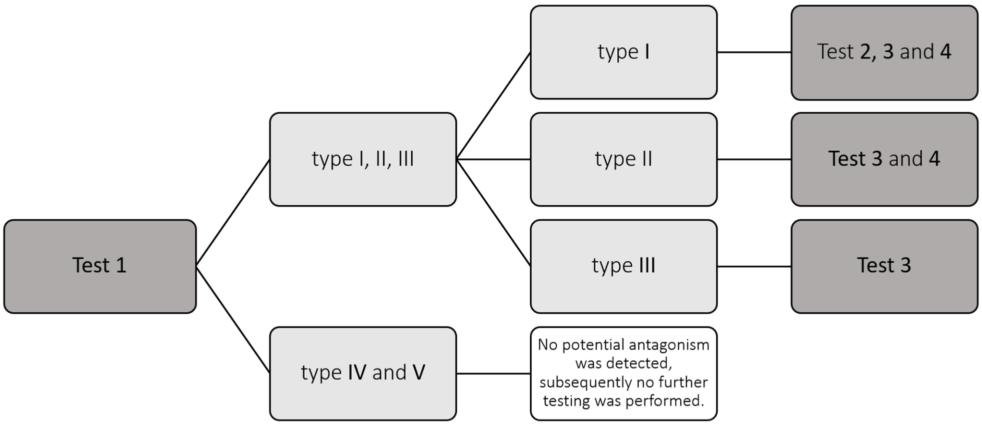
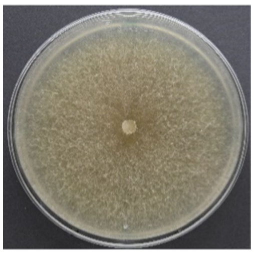
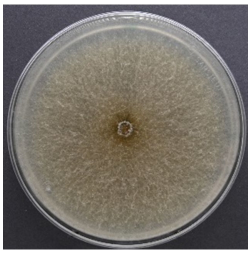
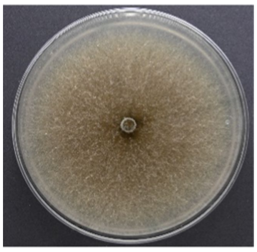


FIGURE 1
Flowchart illustrating the selection of fungal strains for experiments.

TABLE 1 List of *Cryptostroma corticale*-strains, isolated in Germany, used in the dual culture tests.

C. corticale strain number	Referred to as	NCBI accession-No.	Origin of isolation	Isolated from (material)	Culture habitus after 10 days on MYP at room temperature
NW-FVA 5889	CC1	OP010050	Bomstedt, Saxony-Anhalt	Spores from young stem with blisters	
NW-FVA 7011	CC2	PP448053	Hardberg, Hesse	Living woody tissue	
NW-FVA 7148	CC3	PP448054	Dransfeld, Lower-Saxony	Spores from laying wood (bark)	

light, and checked for growth on days three, five, and 10, each measured and marked on the back of the Petri dish. The cultures were measured along the two axes (alpha for the horizontal measurement and beta for the vertical measurement). The measurement stopped once mycelial contact was observed. If an overgrowth of one fungus over the other was observed an "NA" was noted in the measurements for that interaction (Supplementary Table 1). When both fungi met in a deadlock without overgrowth, the same measurements were noted for the following day of measurement since no additional growth was observed. On day 10, pictures were taken with a Nikon D3400 camera of one representative Petri dish out of each three replicates. The picture was later used for visual assessment and placement into the five different types of interaction I—V (Table 3), based on terms defined by Boddy (2000) and Badalyan et al. (2002).

The set-up of Test 1 was repeated after the initial results for all potential antagonists with at least one interaction in type I. The plugs of the potential antagonists were placed on the plate 1 week prior to the inoculation with *C. corticale*. The goal was to see

TABLE 2 **List of all tested strains with taxonomic placement, NCBI accession number, and NW-FVA strain number, and results of Test 1; *Cryptostroma corticale* (CC), CC1: NW-FVA 5889, CC2: NW-FVA 7011 and CC3: NW-FVA 7148.**

Sequenced as	Accession no.	NW-FVA no.	Division[1]	Order[2]	Interaction type based on Test 1		
					CC1	CC2	CC3
Agaricales sp.	ON710962	7192	B	Agaricales	V	V	V
Angustimassarina sp.	ON710906	6253	A	Pleosporales	V	V	V
Angustimassarina sp.	PP448033	6254	A	Pleosporales	V	V	V
Angustimassarina sp.	PP448034	6427	A	Pleosporales	V	V	V
Angustimassarina sp.	PP448035	6587	A	Pleosporales	V	V	V
Apiognomonia sp.	ON710918	6272	A	Diaporthales	V	V	V
Apiospora kogelbergensis	PP448038	7713	A	Amphisphaeriales	IV	IV	V
Apiospora cf. *marii*	ON710953	7004	A	Amphisphaeriales	V	V	IV
Apiospora cf. *marii*	PP448037	7006	A	Amphisphaeriales	V	V	V
Apiospora cf. *marii*	PP448036	7013	A	Amphisphaeriales	IV	IV	IV
Apiospora rasikravindrae	ON710949	6999	A	Amphisphaeriales	V	V	V
Aureobasidium sp.	ON710936	6586	A	Dothideales	V	V	V
Beauveria pseudobassiana	ON710933	6581	A	Hypocreales	I	I	I
Biscogniauxia nummularia	ON710888	6227	A	Xylariales	IV	IV	IV
Boeremia exigua	ON710928	6430	A	Pleosporales	V	V	V
Cadophora prunicola	ON710939	6596	A	Helotiales	V	V	V
Cadophora prunicola	PP448039	6996	A	Helotiales	II	I	I
Calosporella innesii	ON786729	6573	A	Diaporthales	II	II	II
Capronia sp.	ON710913	6262	A	Chaetothyriales	V	V	V
Cephalotrichum sp.	PP448032	6247	A	Hypocreales	V	I	II
Cladosporium sp. 2	ON710963	7195	A	Capnodiales	V	V	V
Cladosporium sp. 3	ON710910	6259	A	Capnodiales	V	V	V
Cladosporium sp. 5	ON710944	6991	A	Capnodiales	V	V	V
Clonostachys rosea	ON710898	6243	A	Hypocreales	II	II	II
coelomycete	DNA extraction failed	7709	A		V	V	II
Coprinellus micaceus	ON710893	6233	B	Agaricales	V	V	V
Coprinellus sp.	ON710950	7000	B	Agaricales	III	III	III
Cytospora cf. *friesii*	ON710902	6249	A	Diaporthales	V	V	V
Cytospora cf. *populina*	ON710895	6237	A	Diaporthales	V	V	V
Cytospora cf. *populina*	PP448055	6240	A	Diaporthales	V	V	V
Cytospora cf. *populina*	PP448056	6242	A	Diaporthales	V	V	V
Cytospora cf. *populina*	PP448042	6421	A	Diaporthales	V	V	V
Cytospora cf. *rodophila*	PP448040	6235	A	Diaporthales	V	V	V
Cytospora cf. *rodophila*	PP448041	6268	A	Diaporthales	V	V	V
Cytospora cf. *rodophila*	PP448057	6269	A	Diaporthales	V	V	V
Diaporthe cf. *eres*	ON710970	7714	A	Diaporthales	IV	V	V
Diaporthe cf. *rudis*	ON710952	7002	A	Diaporthales	V	V	V
Diaporthe cf. *rudis*	PP448043	7012	A	Diaporthales	V	V	V
Diaporthe pustulata	ON710889	6228	A	Diaporthales	II	II	II
Didymella macrostoma	ON710899	6244	A	Pleosporales	V	V	V
Didymella macrostoma	PP448044	6593	A	Pleosporales	V	V	V
Didymellaceae sp.	ON710948	6998	A	Pleosporales	IV	IV	IV

(Continued)

TABLE 2 (Continued)

Sequenced as	Accession no.	NW-FVA no.	Division[1]	Order[2]	Interaction type based on Test 1		
					CC1	CC2	CC3
Dothideomycetes sp.	ON710927	6429	A	i.s.	V	V	V
Dothiorella spp.	ON710891	6230	A	Botryosphariales	V	V	V
Eutypa maura	PP448058	7018	A	Xylariales	IV	IV	IV
Eutypa maura	ON710900	6245	A	Xylariales	IV	IV	IV
Eutypa petrakii var. *hederae*	ON710915	6267	A	Xylariales	V	V	IV
Eutypella quaternata	ON710896	6238	A	Xylariales	II	II	II
Exophiala cf. *pisciphila*	ON710924	6423	A	Chaetothyriales	V	V	V
Hymenochaeta sp.	ON710956	7019	B	Hymenochaetales	V	V	V
Hypholoma fasciculare	ON710897	6241	B	Agaricales	II	I	II
Hypoxylon fragiforme	ON710966	7707	A	Xylariales	IV	V	V
Hypoxylon fragiforme	PP448059	7708	A	Xylariales	IV	IV	IV
Hypoxylon rubiginosum	ON710968	7711	A	Xylariales	V	V	V
Jackrogersella cohaerens	ON710957	7020	A	Xylariales	I	II	I
Leptodontidium sp.	ON710921	6276	A	Helotiales	V	V	V
Leptosillia muelleri	ON710931	6576	A	Xylariales	II	II	II
Lopadostoma turgidum	ON710930	6575	A	Xylariales	V	V	V
Lopadostoma turgidum	PP448045	6607	A	Xylariales	V	V	V
Lophiostoma carpini	ON710946	6993	A	Pleosporales	II	II	II
Lophiostoma carpini	PP448046	6994	A	Pleosporales	V	V	V
Lophium arboricola	ON710911	6260	A	Mytilinidiales	V	V	V
Melanomma populicola	ON710920	6274	A	Pleosporales	V	V	V
Mucoromycota sp.	DNA extraction failed	7022	M		V	V	V
Mycoarthris sp.	ON710925	6426	A	Helotiales	V	V	V
Fungus sp.	DNA extraction failed	6425			V	V	V
Nectria cinnabarina	ON710894	6236	A	Hypocreales	III	III	III
Nectria cinnabarina	PP448047	6239	A	Hypocreales	III	III	III
Neocucurbitaria acerina	ON710909	6258	A	Pleosporales	V	V	V
Neocucurbitaria acerina	PP448048	7204	A	Pleosporales	IV	IV	IV
Neodidymelliopsis sp.	ON710913	6261	A	Pleosporales	V	V	V
Neoleptosphaeria rubefaciens	ON710907	6255	A	Pleosporales	V	V	V
Neonectria cf. *ramulariae*	ON710914	6264	A	Hypocreales	IV	V	IV
Neonectria cf. *ramulariae*	PP448049	6265	A	Hypocreales	V	V	V
Neonectria cf. *ramulariae*	ON786729	6273	A	Hypocreales	IV	IV	IV
Neonectria sp.	ON710934	6582	A	Hypocreales	II	II	II
Neosetophoma cf. *italica*	ON710903	6250	A	Pleosporales	V	V	V
Neosetophoma cf. *samarorum*	PP448050	6277	A	Pleosporales	I	I	I
Nigrograna cf. *norvegica*	ON710960	7189	A	Pleosporales	II	II	II
Nigrograna mycophila	ON710943	6990	A	Pleosporales	V	V	V
Paracamarosporium cf. *fagi*	ON710923	6420	A	Pleosporales	I	II	II
Parapyrenochaeta protearum	ON710942	6989	A	Pleosporales	V	V	V

(Continued)

TABLE 2 (Continued)

Sequenced as	Accession no.	NW-FVA no.	Division[1]	Order[2]	Interaction type based on Test 1		
					CC1	CC2	CC3
Penicillium sp.	PP448051	6257	A	Eurotiales	V	V	V
Petrakia irregularis	ON710932	6580	A	Pleosporales	V	V	V
Pezicula sporulosa	ON710945	6992	A	Helotiales	I	I	I
Pleosporales sp.	ON710922	6278	A	Pleosporales	V	V	V
Porostereum spadiceum	ON710947	6997	B	Polyporales	IV	IV	IV
Preussia cf. *aemulans*	ON710935	6585	A	Pleosporales	I	I	I
Pseudogymnoascus cf. *pannorum*	ON710926	6428	A	Thelebolales	V	V	V
Ramularia collo-cygni	ON710959	7023	A	Mycosphaerellales	V	V	V
Serpula himantioides	ON710967	7710	B	Boletales	II	II	II
Sordariomycetes sp.	ON710969	7712	A		V	V	V
Sordariomycetes sp.	ON710929	6572	A		V	V	V
Stereum cf. *hirsutum*	ON710916	6270	B	Russuales	V	V	V
Thyridium vestitum	ON710904	6251	A	Thyridiales	V	V	V
Tolypocladium sp.	ON710954	7010	A	Hypocreales	II	II	II
Tolypocladium sp.	ON710961	7191	A	Hypocreales	II	II	II
Trametes versicolor	ON710917	6271	B	Polyporales	IV	IV	IV
Trichoderma cf. *gelatinosum*	PP448052	7705	A	Hypocreales	V	V	V
Xenocylindrosporium	ON786728	6592	A	Phaeomoniellales	V	V	V
Xylaria longipes	ON710890	6229	A	Xylariales	V	V	V
Xylariales sp.	DNA extraction failed	6341	A	Xylariales	V	V	V

[1]A, Ascomycota; B, Basidiomycota; M, Mucoromycota; [2]i.s., incertae sedis.

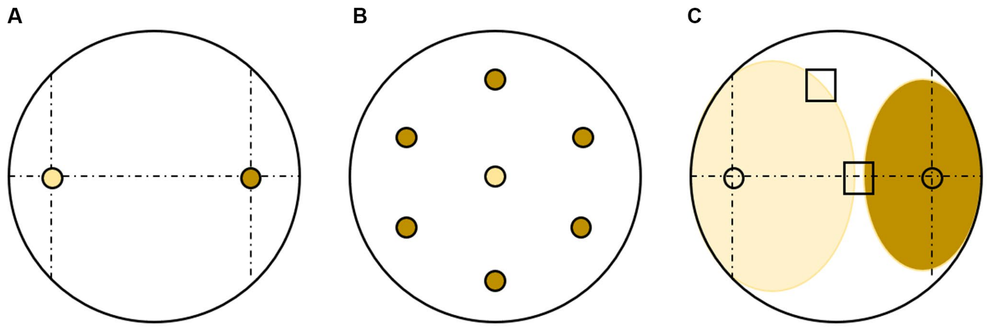


FIGURE 2
Scheme of dual culture tests (a) between pathogen (yellow) and fungal potential antagonist (brown) **(A)** set-up for Test 1 and Test 2 with the pathogen (*Cryptostroma corticale*) on the left and the fungal potential antagonist on the right (Rigerte et al., 2019); **(B)** set-up for pathogen surrounded by six plugs of the fungal potential antagonist in Test 3; **(C)** interaction between pathogen (left) and the potential antagonist (right) for Test 4 with indication of re-isolation spots (Brglez et al., 2020).

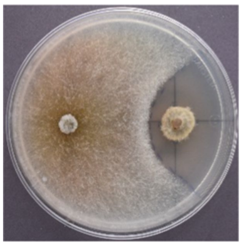
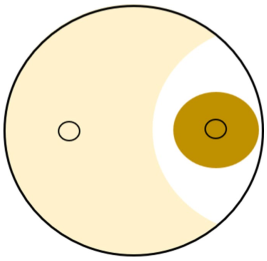
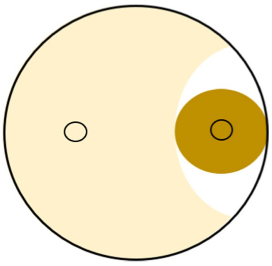
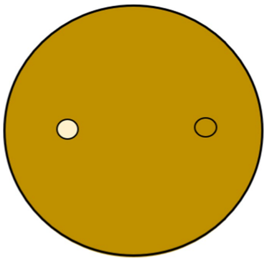
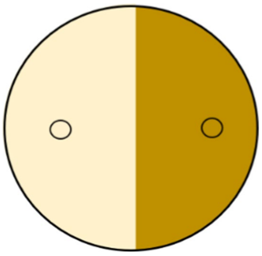
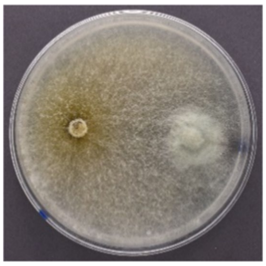
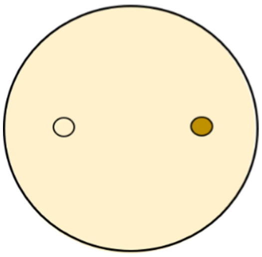
whether the inhibition at a distance would become more prominent, if the potential antagonist had time to establish itself first.

Measuring data of Test 1 was analyzed using RStudio (V. 4.1.2, R Core Team, 2019; Supplementary Table 2) for statistical analysis following the first part of the script by Rigerte et al. (2019). For the R script analysis, the spherical index (alpha/beta) of the fungal growth was calculated. For this approach, it was assumed that a single pathogen on a Petri dish of medium would have a circular growth, i.e., have the same measurements for both alpha and beta, resulting in a spherical index of 1 (Rigerte et al., 2019). If this is true for single cultures, it follows that if the interaction continues in a dual culture setup, alpha and beta would not have the same value, resulting in a spherical index unequal to 1. An inhibition of the pathogen was detected in the data analysis, if the spherical index for the pathogen was smaller than 1 (growth on the alpha axis smaller than on the beta axis) and the spherical index for the endophyte on the dual assay was bigger than 1 (growth on the alpha axis higher than on the beta axis; Rigerte et al., 2019; Figure 3). In this study the control culture was set up with each tested fungus in a pairing against itself, also prepared in triplicates.

2.2 Test 2—Dual cultures on MYP and PDA at 25°C

For specific investigation of the fungi which had at least one interaction against *C. corticale* classified as type I, the set-up of Test 1 was repeated with two different types of media for Test 2, i.e., MYP and PDA at 25°C. For each pairing of *C. corticale* with the respective

TABLE 3 Different types of interaction as defined in this study based on Boddy (2000) and Badalyan et al. (2002) with examples.

Interaction type	Definition	Example	Scheme
I = B	Deadlock without contact	 *C. corticale* × *Pezicula sporulosa*	
II = A	deadlock with contact, Petri dish not fully colonized by *C. corticale*	 *C. corticale* × *Neonectria* sp.	
III = C	replacement of *C. corticale* by the potential antagonist	 *C. corticale* × *Nectria cinnabarina*	
IV = A	deadlock, Petri dish fully colonised, recognition between the fungi visible through barrage	 *C. corticale* × *Eutypa maura*	
V = C	replacement of the potential antagonist by *C. corticale*	 *C. corticale* × *Neocucuritaria acerina*	

"A" deadlock at mycelial contact, "B" deadlock at distance without mycelial contact, and "C" replacement, meaning overgrowth without initial deadlock according to Badalyan et al. (2002).

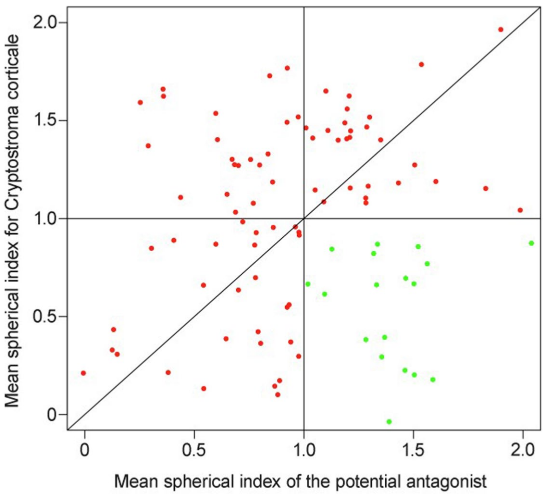


FIGURE 3
Illustration of the mean spherical indices of a set of a fungal endophyte antagonist plotted against their single test pathogen. This is a simulated dataset generated from a random distribution (*n* = 100, mean = 1, standard deviation = 0.5) that mimics empirical data observed in this experiment. Data points colored green are instances with possible antagonistic interaction while red data points indicate non-antagonistic interactions; created with the R-script published by Rigerte et al. (2019, R V4.1.2).

antagonist, three replicates were prepared following the procedure of Test 1. The Petri dishes were stored in a Binder KBW240 climate chamber at 25°C and ambient daylight for 10 days, measured three times, as described for Test 1, and photographed on day 10. In total, nine strains which had at least one interaction classified as type I were tested.

2.3 Test 3—One plug of *C. Corticale* against six plugs of the potential antagonist

The strains tested in Test 3 had at least one interaction classified as type I, II, or III in Test 1 and 2. A total of 22 strains were tested against *C. corticale* (Table 4). An experimental set up was designed where the *C. corticale* plug was placed in the middle of the Petri dish, surrounded by six plugs colonized by the potential antagonist (Figure 2B), which were placed at a distance of 3 cm to *C. corticale*. Strains observed to grow much slower than *C. corticale*, namely *Beauveria pseudobassiana* S.A. Rehner & Humber, *Cadophora prunicola* (strain NW-FVA 6996) Damm & S. Bien, *Calosporella innesii* (Curr.) J. Schröt., *Diaporthe pustulata* Sacc., *Hypholoma fasciculare* (Huds.) P. Kumm., *Jackrogersella cohaerens* (Pers.) L. Wendt, Kuhnert & M. Stadler, *Leptosillia muelleri* (Duby) Voglmayr & Jaklitsch, *Lophiostoma carpini* Andreasen, Jaklitsch & Voglmayr (strain NW-FVA 6993), *Neonectria* sp., *Neosetophoma* cf. *samarorum*, *Paracamarosporium* cf. *fagi*, *Pezicula sporulosa* Verkley, *Preussia* cf. *aemulans*, *Serpula himantioides* (Fr.) P. Karst, *Tolypocladium* sp. (strain NW-FVA 7010 and 7,191) and a coelomycete (NW-FVA 7709) were placed on the test Petri dish 1 week prior to *C. corticale*, to give them time to establish themselves before the inoculation of the fast-growing *C. corticale*. After ten days each interaction was evaluated and again categorized into one of the five interaction types.

2.4 Test 4—Viability test of hyphae

The setup of Test 1 was repeated for Test 4 (see Table 4). Twenty strains with interactions categorized into type I, or II were tested. The aim was to see whether *C. corticale* hyphae were still viable after the hyphal growth on the Petri dish stopped or if the hyphal tips had died and thus stopped growing. After 10 days two areas of the *C. corticale* culture were examined by transferring a 2 mm piece of a single hypha from different spots of the interaction zone. One piece was taken from the side of the interaction zone and one from the middle of the interaction zone, permitted the mycelia had no contact (Figure 2C). The taken pieces were transferred onto an additional new medium Petri dish, respectively, according to Brglez et al. (2020). If the fungi had grown into each other, one sample was taken from each of the two side margins of the culture, where no mycelial contact was observed. The hyphae were sampled under a Stereomicroscope (ZEISS Stemi 508) to ensure that only one hyphal tip was sampled.

We considered those strains as potential antagonists that had the majority of their tested interactions with *C. corticale* placed in type I and the remaining interactions in no less than type II. Potential antagonists, which were considered for further testing were reconsidered after each test. An antagonism was considered "stronger" or "more prominent" when the distance between the two fungal cultures was larger than in a previous test. For some taxa multiple strains were available and tested. Ultimately, fungi which had consistent antagonistic results over all conducted tests were chosen as potential antagonists. The fungi classified as type III were not considered as potential antagonists since the interaction between the two fungi could not be undoubtedly classified.

3 Results

3.1 Test 1—Dual cultures on MYP and statistical analysis

All 102 tested strains, comprising 76 fungal taxa, were grouped as types based on Test 1. The detailed results for the 39 strains classified as type I-IV can be found in Table 4. The remaining 63 strains, which are not shown in this table had all interactions placed into type V and can be found in Table 2. In total 918 interactions were evaluated. For four of the tested taxa a deadlock without contact was observed against all *C. corticale* strains, thus classifying them as type I. These were *B. pseudobassiana*, *N.* cf. *samarorum*, *P. sporulosa*, and *Pr.* cf. *aemulans*. Eleven strains were classified as type II, exhibiting signs of inhibition against *C. corticale*. The strains categorized as type II are *Cal. innesii*, *Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W. Gams, *D. pustulata*, *Eutypella quaternata* (Pers.) Rappaz, *L. muelleri*, *Lo. carpini* (strain NW-FVA 6993), *Neonectria* sp., *Nigrograna* cf. *norvegica*, *S. himantioides*, and *Tolypocladium* sp. (strains NW-FVA 7010 and NW-FVA 7191). Three strains were categorized as type III, *Coprinellus* sp. and two strains of *Nectria cinnabarina* (Tode) Fr. (strains NW-FVA 6236 and NW-FVA 6239). Eleven strains were categorized as type IV, and 63 strains were categorized as type V (see Table 2).

Four of the tested strains, *C. prunicola* (strain NW-FVA 6996), *H. fasciculare*, *Pa.* cf. *fagi*, and *J. cohaerens* showed different interactions with each of the three *C. corticale* strains and were classified as either type I or type II. The remaining 11 strains had to

TABLE 4 Observed interaction types (I-V) in the dual culture of the potential antagonists against *Cryptostroma corticale* for Test 1 and Test 3.

Taxon	NW-FVA ID	Dual culture –Test 1			Growth challenge between *C. corticale* and 6 plugs of the potential antagonist - Test 3			Viability of CC hyphae in -Test 4
		CC1	CC2	CC3	CC1	CC2	CC3	
Apiospora cf. *marii*	7,004	V	V	IV				
Apiospora cf. *marii*	7,013	IV	IV	IV				
Apiospora kogelbergensis	7,713	IV	IV	IV				
*Beauveria pseudobassiana**	6,581	I	I	I	I	I	I	50%
Biscogniauxia nummularia	6,227	IV	IV	IV				
*Cadophora prunicola**	6,996	I	I	II	I	I	I	50%
*Calosporella innesii**	6,573	II	II	II	II	II	II	83%
Cephalotrichum sp.	6,247	V	I	II	V	V	V	100%
Clonostachys rosea	6,243	II	II	II	IV	IV	IV	100%
coelomycete*	7,709	V	V	II	V	V	V	100%
Coprinellus sp.	7,000	III	III	III				
Diaporthe cf. *eres*	7,714	IV	V	V				
*Diaporthe pustulata**	6,228	II	II	II	II	II	II	83%
Didymellaceae sp.	6,998	IV	IV	IV				
Eutypa maura	6,245	IV	IV	IV				
Eutypa maura	7,018	IV	IV	IV				
Eutypa cf. *petrakii* var. *hederae*	6,267	V	V	IV				
Eutypella quaternata	6,238	II	II	II	IV	IV	IV	-[b]
*Hypholoma fasciculare**	6,241	II	I	II	I	I	I	50%
Hypoxylon fragiforme	7,707	IV	V	V				
*Jackrogersella cohaerens**	7,020	I	II	I	I	I	I	50%
*Leptosillia muelleri**	6,576	II	II	II	II	II	V	-[b]
Lophiostoma carpini	6,993	II	II	II	V	V	V	-[b]
Nectria cinnabarina	6,236	III	III	III	III	III	III	
Nectria cinnabarina	6,239	III	III	III	III	III	III	
Neocucurbitaria acerina	7,204	IV	IV	IV				
Neonectria cf. *ramulariae*	6,264	IV	V	IV				
Neonectria cf. *ramulariae*	6,273	IV	IV	IV				
Neonectria sp.*	6,582	II	II	II	IV	IV	I	33%
Neosetophoma cf. *samarorum**	6,277	I	I	I	V	IV	II	100%
Nigrograna cf. *norvegica*	7,189	II	II	II	–[a]	–[a]	–[a]	–[a]
Paracamarosporium cf. *fagi**	6,420	I	II	II	I	I	I	50%
*Pezicula sporulosa**	6,992	I	I	I	I	I	I	50%
Porostereum spadiceum	6,997	IV	IV	IV				
Preussia cf. *aemulans**	6,585	I	I	I	I	I	I	83%
*Serpula himantoides**	7,710	II	II	II	I	I	I	67%
Tolypocladium sp.*	7,191	II	II	II	I	I	I	83%
Tolypocladium sp.*	7,010	II	II	II	I	I	I	100%
Trametes versicolor	6,271	IV	IV	IV				

*The antagonist was placed onto the petri dish 1 week prior to *C. corticale* in Test 3. [a]Strain was not viable anymore when tests were conducted. [b]*C. corticale* overgrew culture of potential antagonist, no sampling of single hyphae was possible. For the tests different *Cryptostroma corticale* (CC) strains were used (CC1: NW-FVA 5889, CC2: NW-FVA 7011 and CC3: NW-FVA 7148), empty cell: not tested; total percentage of viable hyphae for Test 4; strains that were exclusively Type V are not shown.

be placed into more than one category regarding the interactions with the different strains of *C. corticale* (see Tables 2 and 4). For *Cephalotrichum* sp. (NW-FVA 6347) the interactions with the three different strains of *C. corticale* were all placed in different interaction types (CC1 type V, CC2 type I, and CC3 type II).

Ninety-nine of the tested strains had a slower or similar growth rate as *C. corticale* and 61 (type V) of them were overgrown by *C. corticale*. Three species tested in this study had a faster growth than *C. corticale*. Of the 13 taxa with multiple strains tested, only repeat strains of seven taxa were categorized into the same type for their interactions with all three *C. corticale* strains. Six of which were categorized as type V, while *N. cinnabarina* was categorized as type III. For *C. prunicola* strain NW-FVA 6996 the interaction with CC1 was classified as type II and the CC2 and CC3 interactions as type I. In comparison all interactions of *C. prunicola* strain NW-FVA 6596 were categorized as type V. Similar results were observed for *Lo. carpini* strain NW-FVA 6993 and NW-FVA 6994.

The results of the re-tested type I fungi, which were placed on the Petri dish 1 week prior to inoculation with *C. corticale* (Supplementary Table 3) show a more prominent antagonism at a distance for six of the nine tested strains than those of the original Test 1. For those six strains it is visible, that *C. corticale* is unable to grow forwards. This is most prominent for *P. sporulosa* and *Pr.* cf. *aemulans*. For *Cephalotrichum* sp., *Neosetophoma* cf. *samarorum* and *J. cohaerens* no antagonism at a distance could be observed. For *H. fasciculare* an inhibition at a distance could only be observed for CC2 and CC3. CC1 had mycelial contact with the potential antagonist, though the plate was not fully colonized (type II).

According to the results of the analysis of the spherical indices from Test 1 (Figures 4A–C), CC1 expressed a slower growth than against itself in the pairings with four of the potential antagonists. Of these pairings three were classified as type I, while one was classified as type II. CC2 showed a slower growth than in a pairing against itself against five potential antagonists. Four interactions of these pairings were classified as type I, one interaction was classified as type II. Compared to a pairing against itself, CC3 showed a slower growth when paired against six of the potential antagonists. Out of these only three interactions were classified as type I, the remaining interactions were classified as type II.

In total 19 strains were classified as type I, type II, or a combination of the two, and subsequently as potential antagonists to be investigated in the following tests (Table 4).

3.2 Test 2—Dual culture on MYP and PDA at 25°C

In Test 2, the fungi that previously exhibited an interaction classified as type I against at least one *C. corticale* strain, showed variation between types of media. The antagonistic reaction was more prominent and stronger on PDA than on MYP at a temperature of 25°C for all stains except *Cephalotrichum* sp. (NW-FVA 6247; see Supplementary Table 3). After the tenth day of incubation at 25°C, differences in growth rate and observed antagonism on MYP could be observed for some of the tested fungi compared to the tests at room temperature (see Table 5 and Supplementary Table 3). Three strains had a visibly slower growth at 25°C than at room temperature against themselves. The interactions between *H. fasciculare* and CC2 and CC3 were more prominent at 25°C. In both, *N.* cf. *samarorum* and *Pa.* cf. *fagi*, all combinations had mycelial contact without the Petri dish being fully colonized, so they were classified as type II and not as type I as at room temperature. For *Pr.* cf. *aemulans* all interactions were classified as type I, confirming the results of Test 1. However, the *C. corticale* cultures stopped growing in a straight line at 25°C, while those at room temperature continued to colonize the Petri dish, growing in a half circle, as shown in Table 3 for type I. *Cephalotrichum* sp. did not show an antagonistic reaction against CC2 in Test 2 on neither of the tested media and interacted according to type V, not confirming the observations from Test 1.

3.3 Test 3—One plug of *C. Corticale* against six plugs of the potential antagonist

For three out of the four fungi classified as type I, the initially observed interaction type from Test 1 was confirmed in Test 3 (Table 4). For *N.* cf. *samarorum* the interaction type could not be confirmed for CC2 in this test, for CC3 the interaction type appears to be confirmed for four of the six mycelium plugs placed. In this interaction, *C. corticale* was able to advance to mycelial contact with the remaining two mycelium plugs, but was not able to overgrow them during the 10-day observation. Out of the 11 type II fungi the growth challenge was conducted for 10. *Nigrograna* cf. *norvegica* was not viable anymore at the time of testing. Out of these 10 the interaction for three strains, *S. himantoides*, and cf. *Tolypocladium* sp. (strains NW-FVA 7010 and 7,191), could be classified as type I and the interaction according to type II was confirmed for two other strains (*Cal. innesii* and *D. pustulata*). The interaction in the growth challenge was classified into type IV for *Clonostachys rosea* and *E. quaternata*, and into type V for *Lo. carpini*. For the three remaining type II strains tested, the results differed among the three *C. corticale* strains. *C. prunicola* strain NW-FVA 6996, *H. fasciculare* and *P.* cf. *fagi* had their interactions in Test 3 classified into type I, respectively, as opposed to a mix of type I and II in Test 1. Strain NW-FVA 7709 (coelomycete), as well as *Cephalotrichum* sp. had all three interactions of Test 3 were classified into type V, thus not showing any antagonistic interaction. For both *N. cinnabarina* strains the reaction according to type III was confirmed.

3.4 Test 4—Viability test of hyphae

Of the 102 hyphal samples taken from *C. corticale*, 74 (73%) were still viable. From five of the 20 potentially antagonistic strains tested, all the examined hyphae of *C. corticale* were still able to grow after being transferred to a new MYP-Petri dish (Tables 4 and 6). For the interactions between the three *C. corticale* strains and *H. fasciculare* only the samples taken from the side were viable. Out of the four type I fungi, all six samples taken from *N.* cf. *samarorum* were taken from the side and were still viable. Whereas for both *B. pseudobassiana* and *P. sporulosa* one sample from the middle and one sample from the side margin of every Petri dish was taken, while only the samples from CC1 were both still viable. For both of these strains one of the CC2 samples was still viable, for *B. pseudobassiana* the sample from the side margin and for *P. sporulosa* the sample taken from the middle. All four samples taken from the interaction with CC3 were not viable anymore. Five out of six samples taken from *Pr.* cf. *aemulans* were still viable. Here, also equal samples of middle and side margin were taken.

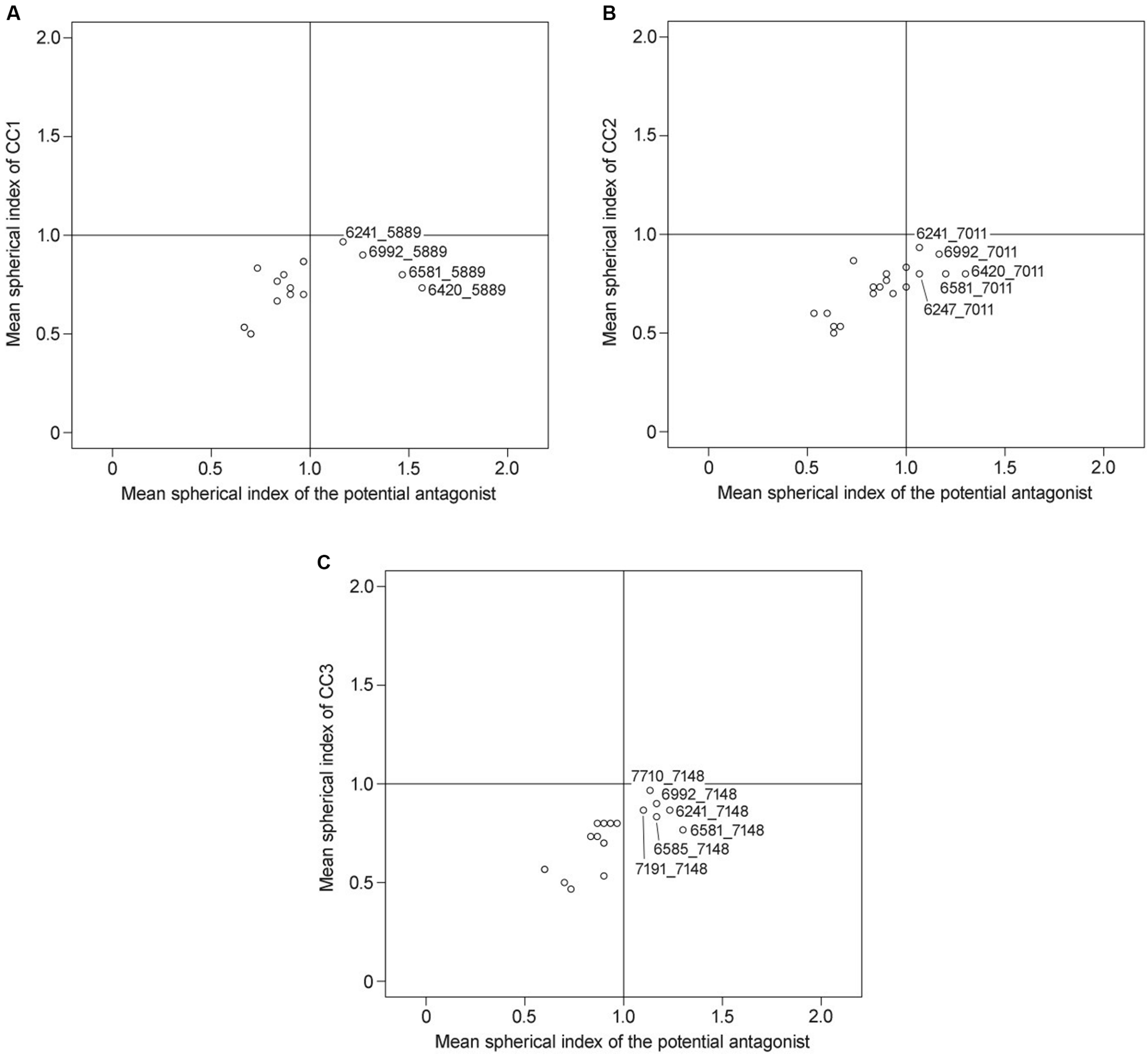


FIGURE 4
Results of the of the mean spherical indices from Test 1 shown for the day 10 measurements, separated by *Cryptostroma corticale* strain showing the interactions with potential antagonism in the lower right quadrant, *Cryptostroma corticale* (CC), **(A)** CC1: NW-FVA 5889, **(B)** CC2: NW-FVA 7011, **(C)** CC3: NW-FVA 7148. The potentially antagonistic interactions for each strain are labeled with the individual interaction abbreviations (NW-FVA number antagonist_NW-FVA number *C. corticale* strain).

The only sample not viable anymore from this strain was the sample taken from the middle of the interaction with CC1. No samples of *C. corticale* could be taken from pairings with *E. quaternata*, *L. muelleri* and *Lo. carpini*, because *C. corticale* had overgrown the potential antagonist.

4 Discussion

Throughout the experimentation process, the number of potential antagonists identified in Test 1 decreased with each subsequent test. Ultimately, strains of five fungal species demonstrated the most consistent potential as antagonists against *C. corticale* due to their inhibitory effects across all four tests, and will be discussed hereinafter.

Hypholoma fasciculare commonly colonizes logs of beech wood, as well as the wood of other broad-leaved tree species (Coates and Rayner, 1985) and has also been reported as a saprophyte causing white rot in roots and tree trunks (Folman et al., 2008; Šnajdr et al., 2011). It has previously been reported that *H. fasciculare* possesses antagonistic properties against other fungi (e.g., Badalyan et al., 2002) and has already been described as a potential BCA against *Armillaria ostoyae* Romagn. (Herink) in North America (Chapman and Xiao, 2000; Stevens et al., 2020). Species of the genus *Hypholoma* can also be found in the soil and decomposing litter in forests (Thompson et al., 2012).

Jackrogersella cohaerens, as well as many other fungi belonging to the family of the Hypoxylaceae (Wendt et al., 2018), are known to be common endophytes of woody tissue, and can be beneficial to the host (Halecker et al., 2020; Langer and Bußkamp, 2021; Song et al.,

TABLE 5 Results of Test 2 shown for comparison for nine promising potential fungal antagonists fungi identified in Test I and II; MYP: Malt Yeast Peptone Agar, PDA: Potato Dextrose Agar, *Cryptostroma corticale* (CC), CC1: NW-FVA 5889, CC2: NW-FVA 7011 and CC3: NW-FVA 7148.

Name	NW-FVA number	CC1			CC2			CC3		
		MYP	MYP 25°C	PDA 25°C	MYP	MYP 25°C	PDA 25°C	MYP	MYP 25°C	PDA 25°C
Beauveria pseudobassiana	6581	I	II	II	I	II	II	I	II	II
Cadophora prunicola	6996	II	V	II	I	V	II	I	II	I
Cephalotrichum sp.	6247	V	V	V	I	V	V	II	V	V
Hypholoma fasciculare	6241	II	II	I	I	I	I	II	I	I
Jackrogersella cohaerens	7020	I	I	I	II	I	I	I	I	I
Neosetophoma cf. *samarorum*	6277	I	II	II	I	II	II	I	II	II
Paracamarosporium cf. *fagi*	6420	I	II	I	II	II	I	II	II	I
Pezicula sporulosa	6992	I	II	I	I	I	I	I	I	I
Preussia cf. *aemulans*	6585	I	I	I	I	I	I	I	I	I

TABLE 6 Results of Test 4, where the viability of hyphal samples was tested; *Cryptostroma corticale* (CC), CC1: NW-FVA 5889, CC2: NW-FVA 7011, CC3: NW-FVA 7148.

	No. of hyphal samples taken from the middle of the *C. corticale* culture	Viable (%)	No. of hyphal samples taken from the side margin of the *C. corticale* culture	Viable (%)	Hyphal samples viable in total
CC1	9	4 (44%)	25	19 (76%)	23 (68%)
CC2	11	7 (64%)	23	21 (91%)	28 (82%)
CC3	10	2 (20%)	24	21 (88%)	23 (68%)
Sum	30	13 (43%)	72	61 (85%)	74 (73%)

2022). *Jackrogersella cohaerens* primarily colonizes dead or dying beech wood and can be a saprophyte on beech (Sinclair and Lyon, 2005). It has been described as a colonizer of beech and less frequently maple (Sinclair and Lyon, 2005), and can cause disease in beech trees (Schumacher et al., 2006). As *J. cohaerens* can cause disease on beech, it would be important to test whether it could potentially cause disease on sycamore as well, especially since the strain tested was isolated from discolored and decaying wood of *A. pseudoplatanus* by Schlößer et al. (2023).

Paracamarosporium cf. *fagi* is a fungus belonging to the Pleosporales. The first report was made from twigs of *Fagus sylvatica* L. (Crous et al., 2015). It is a Coniothyrium-like species and is described to also appear on *Betula pendula* Roth, *Elaeagnus rhamnoides* L., and was found saprobic on twigs of *Ziziphus jujube* Mill (Hyde et al., 2020). It has been reported from Germany, the Republic of Latvia and Ukraine (Crous et al., 2015; Hyde et al., 2020). Other fungi of the genus *Coniothyrium*, i.e., *Coniothyrium minitans*, have been described as potential BCAs (e.g., Whipps and Gerlagh, 1992; De Vrije et al., 2001).

Pezicula sporulosa has been described as an endophyte on *Abies beshanzuensis* Wu. (Yuan et al., 2011). Other *Pezicula* species also appear as endophytes in several different coniferous and broadleaved trees (Noble et al., 1991; Kehr, 1992; Kowalski and Kehr, 1992; Schulz et al., 1995; Langer et al., 2021). Simultaneously, *P. sporulosa* can cause canker disease on various species of coniferous and broadleaved trees in Europe (Rossman et al., 2018). Nevertheless, several *Pezicula* species possess antifungal properties (Noble et al., 1991; Schulz et al., 1995). A recent study showed that a strain of *Pezicula* cf. *ericae* has antagonistic properties against the causal agent of ash dieback, *Hymenscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya (Demir et al., 2023).

Preussia aemulans (Rehm) Arx is known as an endophyte and saprotroph (Domsch et al., 1980 as cited in Elmholt and Kjøller, 1989; Gonzalez-Menendez et al., 2017). The genus *Preussia* contains several endophytic species (Arenal et al., 2007; Porras-Alfaro et al., 2014; Massimo et al., 2015) but is also generally known to occur on leaf litter, dung or in soil (Cain, 1961; Rai and Tewari, 1963; Peterson et al., 2009; Asgari and Zare, 2010). Some strains of the genus *Preussia* have also been described to have antifungal/antimicrobial properties (Weber and Gloer, 1988, 1991; Mapperson et al., 2014; Motlagh and Usefipoor, 2016; Perlatti et al., 2021).

4.1 Test 1—Dual cultures on MYP and statistical analysis

Test 1 revealed that *C. corticale* often overgrew potential antagonists due to its rapid growth compared to most fungi.

Given that *C. corticale* has a higher growth rate than the majority of fungi in our study, it appears to have an advantage over these slow-growing fungi in terms of colonizing tissue. Six of the re-tested type I strains, which were placed on the Petri dish 1 week prior to *C. corticale*, exhibited a more prominent antagonism than observed in the original Test 1. This could indicate that organic compounds were released into the growth media by the potential antagonists, creating a stronger antagonistic reaction (Boddy, 2016). Similar results were demonstrated by Sonnenbichler et al. (1994) and recently shown for antagonism assays between *H. fraxineus* and an endophyte isolated from *F. excelsior* by Demir et al. (2023). This information can be used for further investigation into a potential control for *C. corticale*.

The majority of the fungi tested showed the same response in the three replicates and pairings with the three different *C. corticale* strains. However, for some tested fungal strains one or more of the replicates showed different results and the results varied strongly between the three *C. corticale* strains. The various interactions with the different *C. corticale* strains were therefore categorized into different interaction types, as evident for *Cephalotrichum* sp. (NW-FVA 6247). It has previously been observed, that the fungus-fungus interaction can vary in some cases between the same fungi, even under seemingly identical conditions (Rayner et al., 1994; Boddy, 2000; Brglez et al., 2020). This might be a factor in the differences observed between the interactions with the individual *C. corticale* strains.

The statistical analysis of the measurements of Test 1 simply represents the growth of each individual fungus by its respective spherical index. Consequently, the data analysis does not reflect the interaction between the fungi, i.e., replacement or deadlock. The spherical index of the fungal growth does not account for the space left in between the fungi, since it only analyses the growth of one of the two fungi. This aspect was also critically stated by Rigerte et al. (2019) in the original methodology. Observation of distance left between the tested fungi on the last day of measurement was a valuable characterization for detecting potential antagonisms, allowing us to detect 19 potential antagonists (type I and II) from Test 1.

4.2 Test 2—Dual culture on MYP and PDA at 25°C

In Test 2, *C. corticale* appeared to be more competitive growing on MYP at 25°C, its optimal growing temperature (Dickenson, 1980), than at room temperature. For all but one strain the interaction was more prominent on PDA than on MYP at 25°C. As described in several other studies, different types of artificial media affect the growth rates and habitus of fungal cultures (Botella et al., 2016), mycelial interactions between fungi (Brasier and Webber, 2013) as well as the production of antibiotics and a fungus' response to these antibiotics (Whipps, 1987). These findings account for differences in interaction as well as variance in the morphology of fungi on different artificial media, as observed for *J. cohaerens*. Furthermore, it was observed that some fungi, which were originally classified as interaction type II in Test 1, were classified into type I in Test 2. On the other hand, a switch from interaction type I to type II was also observed. The different behavior is likely a result of the different temperature, as compared to Test 1.

4.3 Test 3—One plug of *C. Corticale* against six plugs of the potential antagonist

Test 3 clarified the previous observations made in Test 1 and 2 (Table 4 and Supplementary Table 3). No antagonism was observed for coelomycete (NW-FVA 7709) in Test 3, but interestingly, the culture habitus of *C. corticale* in this pairing differed from its normal appearance in pure culture. This suggests that there was some kind of interaction between the coelomycete and *C. corticale* altering the mycelial growth of the latter. Similar observations have been made by Donnelly and Boddy (2001) for *H. fasciculare* and *Agrocybe gibberosa* (Fr.) Fay. when placed against *Stropharia caerulea* (Kriesel). *Cephalotrichum* sp. (NW-FVA 6247) was the only fungus where all three interactions with the different *C. corticale* strains were placed into different types in this study, and no antagonism could be observed in Test 3. All interactions from Test 3 for *Cephalotrichum* sp. were classified as type V, showing no antagonistic properties against any of the tested *C. corticale* strains. One possible explanation for these inconsistent results observed from *Cephalotrichum* sp. is that there is a difference between the three *C. corticale* isolates used in Test 1, which only specifically affects *Cephalotrichum* sp. Another possible explanation for the change in behavior of *Cephalotrichum* sp. might be the fact that Test 1 and 2 were conducted the same year the fungi were isolated. Test 3 and 4 were conducted one and a half years later, after the fungi had been stored in the fungal collection in tubes with MYP media at +4°C. As Humber (1997) and Butt and Copping (2000) state, fungi kept on artificial medium, especially after serial transfer, can lose their antagonistic characteristics, as observed for six of the tested strains. To reduce the loss of competitiveness and antagonistic properties of fungi, one solution is to periodically change the growth medium or even periodically transfer the fungus to suitable sterile host material (Marx and Daniel, 1976; Thomson et al., 1993). As a standard procedure conducted in this study, a transfer between different nutrient media was done for each strain after taking them out of the collection. After being stored on MYP the fungi were transferred to Malt-Extract-Agar according to Bußkamp (2018) for a week and then transferred back to MYP to prepare the cultures for the experiment. Nevertheless, this does not exclude the loss or weakening of certain traits (Tables 5, 6).

4.4 Test 4—Viability test of hyphae

In Test 4 it was found that 73% of the tested hyphae of *C. corticale* were capable of growth when re-cultivated on new artificial medium, even though *C. corticale* no longer showed growth in the original dual culture with the potential antagonist. Similar results were reported by Brglez et al. (2020), who found that after deadlock at distance the sampled hyphae of the tested pathogen, *E. quaternata* (Pers.) Rappaz, grew out eight out of 10 times. This indicates that although the growth of *C. corticale* was inhibited by the antagonist, it is still viable and able to colonize fresh nutrient medium, suggesting a fungistatic effect. Possibly, there were inaccuracies in cutting the hyphal pieces from the dual culture when extracting dead hyphal cells next to still living hyphae. However, it is possible that through organic compounds emitted by the potential antagonist, the pathogen might be inhibited in its growth and thus unable to colonize the uncultured media. Baptista et al. (2021), for example, state that many of the secondary metabolites produced by *H. fasciculare* are known to have antimicrobial effects. Also for *P. sporulosa* the production of antifungal secondary metabolites is known (McMullin et al., 2017). Some of these secondary metabolites can cause mortality of fungi, as described for a combination of beauvericin and ketoconazole against *Candida parapsilosis* (Ashford) Langeron & Talice by Wang and Xu (2012).

4.5 General discussion

Dual culture tests on artificial media, such as those used in this study, are useful for observing interactions between fungi, but do not consider the complexity of interactions *in vivo*. The results of antagonism tests have been shown to differ significantly between *in vitro* and *in vivo* antagonism tests (Dowson et al., 1988; Woods et al., 2005; Peters et al., 2020). The same applies to the differences between greenhouse and field trials (Heydari and Pessarakli, 2010). Still, *in vitro* antagonism tests are necessary and useful to gain an insight into fungal interactions previous to *in vivo* tests. The growing rate *in vivo* might differ from the growing rate *in vitro* for both *C. corticale* and the potential antagonists. The growing rate of *C. corticale in vivo* is insufficiently researched. The results obtained by Alcock and Wheeler (1983) show though, that the *in vivo* growth rate of *C. corticale* appears to depend more on the host factor than the isolate itself. This makes it hard to predict the *in vivo* interaction, which can differ quite significantly from *in vitro* observations (e.g., Lundborg and Unestam, 1980; Raziq and Fox, 2003).

One possible explanation for the observed inconsistencies in antagonistic behavior, as described above for, e.g., *Cephalotrichum* sp., is that most of the tested fungi might utilize a different carbon source than *C. corticale* and additionally might not naturally be antagonistic. Oliva et al. (2021) showed that the mycobiome of pine trees changes after hail damage and demonstrated that different fungi grow differently on different media, suggesting that they use different carbon sources. Another factor that may be relevant is that *C. corticale* is not a native fungus of Central Europe. Due to its recent introduction in an evolutionary time frame and the lack of time for coevolution with other fungi (Prospero et al., 2021) or with the host species itself, it is possible that there are no true natural antagonists in Europe despite its presence in Germany since the 1960s. The observation that *C. corticale* overgrew most of the tested fungi adds to the assumption that it is very competitive, underlining the need for an effective and strong antagonist.

It would be necessary to investigate if *H. fasciculare*, *J. cohaerens*, *Pa*. cf. *fagi*, *P. sporulosa*, and *Pr.* cf. *aemulans* can cause symptoms on *A. pseudoplatanus* and, if so, what kind of symptoms are caused. Following the results of such an experiment, it could be decided whether the infection with the potential antagonist is more beneficial than with *C. corticale*. Considering that *H. fasciculare* can cause white rot, it is not an ideal candidate for a BCA. As an alternative to using a living organism, which can be unpredictable and could cause symptoms itself, it could be beneficial to get an understanding of the secondary metabolome of the five potential antagonists identified in this study. It is possible that secondary metabolites play a role in the inhibition of *C. corticale* (Woodward and Boddy, 2008). Such organic compounds with antimicrobial and antifungal properties have been identified for two of the five potential antagonists, namely *H. fasciculare* and *P. sporulosa* (McMullin et al., 2017; Baptista et al., 2021). For a wide use as a BCA, it is relevant for the potential antagonist to be effective against any strain of *C. corticale*, which could be encountered in a plant. This could also be resolved by using secondary metabolites instead of living organisms. Since trees are perennial plants, as opposed to annual crops, it would be beneficial for a biological control measure for trees to be effective over several years. Depending on the pathogen, the development of a disease in woody tissues may take longer than one growing season. Since many pathogens persist in the host for several years (Prospero et al., 2021) they require long-term control. It is also important to consider the time of year a pathogen first infects the plant, the plant tissue the pathogen mainly infects, and how the interaction between pathogen and antagonist develops in time and space (Heydari and Pessarakli, 2010). The time of plant infection is crucial for the successful establishment of the pathogen within the plant (Rodriguez and Redman, 1997). For biological control to be effective, the antagonistic fungus has to establish itself in the plant in order to be effective as a preventative rather than a curative measure (Heydari and Pessarakli, 2010). Several factors have to be considered for a biological control in a forest pathosystem in comparison to the application in agricultural systems, e.g., a more complex anatomy of trees than crops, a longer lifespan of trees, and a lack of regular plant rotation, which can reduce the levels of potential inoculum (Cazorla and Mercado-Blanco, 2016). Treating forest stands with biological control agents could be more challenging than treating trees in urban settings. The number of plants that would need to be treated alone poses a challenge in terms of manpower and the potential for periodic treatment renewal.

5 Conclusion and outlook

This study can provide a basis for further research into potential antagonists against *C. corticale*. Out of the 102 fungal strains associated with sycamore tested, we were able to identify five promising potential antagonists against *C. corticale*, namely *H. fasciculare*, *J. cohaerens*, *Pa.* cf. *fagi*, *Pr.* cf. *aemulans*, and *P. sporulosa*. These fungi appear to have a certain potential as biological controls *in vitro*. Prior to *in planta* experiments with both *C. corticale* and the potential antagonist, pathogenicity tests with the potential antagonists should be carried out on *A. pseudoplatanus* to ensure their harmlessness to the tree when used in an infection set-up. The next step, in order to find biological control against *C. corticale*, should be to test these most promising antagonists *in planta*, e.g., in greenhouse experiments. This could be done by using dual infections in young sycamore trees to see if the responses observed *in vitro* could be extrapolated to the *in vivo* interaction in host plants.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Author contributions

RS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing, Writing – original draft. SB:

Conceptualization, Methodology, Writing – review & editing. JB: Conceptualization, Methodology, Writing – review & editing. GL: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing. EL: Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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Supplementary material

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References

Alcock, S., and Wheeler, B. E. J. (1983). Variability of Cryptostroma corticale, the causal fungus of sooty bark disease of sycamore. *Plant Pathol.* 32, 173–176. doi: 10.1111/j.1365-3059.1983.tb01316.x

Arenal, F., Platas, G., and Pelaez, F. (2007). A new endophytic species of *Preussia* (Sporormiaceae) inferred from morphological observations and molecular phylogenetic analysis. *Fungal Divers.* 25, 1–17.

Asgari, B., and Zare, R. (2010). Two new species of *Preussia* from Iran. *Nova Hedwigia* 90, 533–548. doi: 10.1127/0029-5035/2010/0090-0533

Badalyan, S. M., Innocenti, G., and Garibyan, N. G. (2002). Antagonistic activity of xylotrophic mushrooms against pathogenic fungi of cereals in dual culture. *Phytopathol. Mediterr.* 41, 220–225. doi: 10.1400/14513

Baptista, P., Guedes De Pinho, P., Moreira, N., Malheiro, R., Reis, F., Padrão, J., et al. (2021). *In vitro* interactions between the ectomycorrhizal *Pisolithus tinctorius* and the saprotroph *Hypholoma fasciculare* fungi: morphological aspects and volatile production. *Mycology* 12, 216–229. doi: 10.1080/21501203.2021.1876778

Bastos, A., Fu, Z., Ciais, P., Friedlingstein, P., Sitch, S., Pongratz, J., et al. (2020). Impacts of extreme summers on European ecosystems: a comparative analysis of 2003, 2010 and 2018. *Philos. Trans. R. Soc. B* 375:20190507. doi: 10.1098/rstb.2019.0507

Boddy, L. (2000). Interspecific combative interactions between wood-decaying basidiomycetes. *FEMS Microbiol. Ecol.* 31, 185–194. doi: 10.1111/j.1574-6941.2000.tb00683.x

Boddy, L. (2016). "Interactions between Fungi and other microbes" in The Fungi. eds. M. J. Carlile, S. C. Watkinson and G. W. Gooday (Amsterdam: Elsevier), 337–360.

Botella, L., Čermáková, V., Bačová, A., and Dvořák, M. (2016). ADA, a fast-growth medium for Hymenoscyphus fraxineus. *For. Pathol.* 46, 85–87. doi: 10.1111/efp.12251

Brasier, C., and Webber, J. (2013). Vegetative incompatibility in the ash dieback pathogen *Hymenoscyphus pseudoalbidus* and its ecological implications. *Fungal Ecol.* 6, 501–512. doi: 10.1016/j.funeco.2013.09.006

Braun, M., Klingelhöfer, D., and Groneberg, D. A. (2021). Sooty bark disease of maples: the risk for hypersensitivity pneumonitis by fungal spores not only for woodman. *J. Occup. Med. Toxicol.* 16:2. doi: 10.1186/s12995-021-00292-5

Brglez, A., Piškur, B., and Ogris, N. (2020). *In vitro* interactions between *Eutypella parasitica* and some frequently isolated fungi from the wood of the dead branches of young sycamore maple (*Acer pseudoplatanus*). *Forests* 11:1072. doi: 10.3390/f11101072

Busby, P. E., Ridout, M., and Newcombe, G. (2016). Fungal endophytes: modifiers of plant disease. *Plant Mol. Biol.* 90, 645–655. doi: 10.1007/s11103-015-0412-0

Bußkamp, J. (2018). Schadenserhebung, Kartierung und Charakterisierung des Diplodia-Triebsterbens der Kiefer, insbesondere des endophytischen Vorkommens in den klimasensiblen Räumen und Identifikation von den in Kiefer (*Pinus sylvestris*) vorkommenden Endophyten. Kassel: Universität Kassel. Available at: https://urn.fi/urn:nbn:de:hebis:34-2018092156417

Butt, T. M., and Copping, L. G. (2000). Fungal biological control agents. *Outlook* 11, 186–191. doi: 10.1039/b008009h

Cain, R. F. (1961). Studies of coprophilus ascomycetes VII. Preussia. *Can. J. Bot.* 39, 1633–1666. doi: 10.1139/b61-144

Cazorla, F. M., and Mercado-Blanco, J. (2016). Biological control of tree and woody plant diseases: an impossible task? *BioControl* 61, 233–242. doi: 10.1007/s10526-016-9737-0

Chapman, B., and Xiao, G. (2000). Inoculation of stumps withHypholomafasciculareas a possible means to control armillaria root disease. *Can. J. Bot.* 78, 129–134. doi: 10.1139/b99-170

Coates, D., and Rayner, A. D. M. (1985). Fungal population and community development in cut beech logs: I. Establishment via the aerial cut surface. *New Phytol.* 101, 153–171. doi: 10.1111/j.1469-8137.1985.tb02823.x

Crous, P. W., Schumacher, R. K., Wingfield, M. J., Lombard, L., Giraldo, A., Christensen, M., et al. (2015). Fungal systematics and evolution: FUSE 1. *Sydowia* 67, 81–118. doi: 10.12905/0380.sydowia67-2015-0081

De Vrije, T., Antoine, N., Buitelaar, R. M., Bruckner, S., Dissevelt, M., Durand, A., et al. (2001). The fungal biocontrol agent *Coniothyrium minitans*: production by solid-state fermentation, application and marketing. *Appl. Microbiol. Biotechnol.* 56, 58–68. doi: 10.1007/s002530100678

Demir, Ö., Zeng, H., Schulz, B., Schrey, H., Steinert, M., Stadler, M., et al. (2023). Bioactive compounds from an endophytic *Pezicula* sp. showing antagonistic effects against the ash dieback pathogen. *Biomol. Ther.* 13:1632. doi: 10.3390/biom13111632

Desprez-Loustau, M.-L., Robin, C., Reynaud, G., Déqué, M., Badeau, V., Piou, D., et al. (2007). Simulating the effects of a climate-change scenario on the geographical range and activity of forest-pathogenic fungi. *Can. J. Plant Pathol.* 29, 101–120. doi: 10.1080/07060660709507447

Dickenson, S. J. (1980). Biology of *Cryptostroma corticale* and the sooty bark disease of sycamore. Ascot: University of Bath.

Dickenson, S., and Wheeler, B. E. J. (1981). Effects of temperature, and water stress in sycamore, on growth of *Cryptostroma corticale*. *Trans. Br. Mycol. Soc.* 76, 181–185. doi: 10.1016/S0007-1536(81)80136-2

Domsch, K. H., Gams, W., and Anderson, T. H. (1980). Compendium of soil Fungi, vol. *1-2*. London: Academic Press.

Donnelly, D. P., and Boddy, L. (2001). Mycelial dynamics during interactions between *Stropharia caerulea* and other cord-forming, saprotrophic basidiomycetes. *New Phytol.* 151, 691–704. doi: 10.1046/j.0028-646x.2001.00211.x

Dowson, C. G., Rayner, A. D. M., and Boddy, L. (1988). The form and outcome of mycelial interactions involving cord-forming decomposer basidiomycetes in homogeneous and heterogeneous environments. *New Phytol.* 109, 423–432. doi: 10.1111/j.1469-8137.1988.tb03718.x

Eastburn, D. M., McElrone, A. J., and Bilgin, D. D. (2011). Influence of atmospheric and climatic change on plant–pathogen interactions. *Plant Pathol.* 60, 54–69. doi: 10.1111/j.1365-3059.2010.02402.x

Ellis, J. B., and Everhart, B. M. (1889). New species of hyphomycetous fungi. *J. Mycol.* 5, 68–72. doi: 10.2307/3752309

Elmholt, S., and Kjøller, A. (1989). Comparison of the occurrence of the saprophytic soil Fungi in two differently cultivated field soils. *Biol. Agric. Hortic.* 6, 229–239. doi: 10.1080/01448765.1989.9754520

Enderle, R., Riebesehl, J., Becker, P., and Kehr, R. (2020). "Rußrindenkrankheit an Ahorn - Biologie, Pathologie und Entsorgung von Schadholz" in Jahrbuch der Baumpflege 2020. ed. D. Dujesiefken (Braunschweig: Haymarket Media), 85–100.

Folman, L. B., Klein Gunnewiek, P. J. A., Boddy, L., and De Boer, W. (2008). Impact of white-rot fungi on numbers and community composition of bacteria colonizing beech wood from forest soil. *FEMS Microbiol. Ecol.* 63, 181–191. doi: 10.1111/j.1574-6941.2007.00425.x

Gonzalez-Menendez, V., Martin, J., Siles, J. A., Gonzalez-Tejero, M. R., Reyes, F., Platas, G., et al. (2017). Biodiversity and chemotaxonomy of *Preussia* isolates from the Iberian Peninsula. *Mycol. Progress* 16, 713–728. doi: 10.1007/s11557-017-1305-1

Gregory, P. H., and Waller, S. (1951). *Cryptostroma corticale* and sooty bark disease of sycamore (*Acer pseudoplatanus*). *Trans. Br. Mycol. Soc.* 34, 579–IN10. doi: 10.1016/S0007-1536(51)80043-3

Greig, B. J. W. (1976). Biological control of *Fomes annosus* by *Peniophora gigantea*. *Forest Pathol.* 6, 65–71. doi: 10.1111/j.1439-0329.1976.tb00508.x

Halecker, S., Wennrich, J.-P., Rodrigo, S., Andrée, N., Rabsch, L., Baschien, C., et al. (2020). Fungal endophytes for biocontrol of ash dieback: the antagonistic potential of *Hypoxylon rubiginosum*. *Fungal Ecol.* 45:100918. doi: 10.1016/j.funeco.2020.100918

Hänsel, S., Hoy, A., Brendel, C., and Maugeri, M. (2022). Record summers in Europe: variations in drought and heavy precipitation during 1901–2018. *Int. J. Climatol.* 42, 6235–6257. doi: 10.1002/joc.7587

Harman, G. E. (1991). Seed treatments for biological control of plant disease. *Crop Prot.* 10, 166–171. doi: 10.1016/0261-2194(91)90038-S

Harman, G. E., and Taylor, A. G. (1988). Improved seedling performance by integration of biological control agents at favorable pH levels with solid matrix priming. *Phytopathology* 78:520. doi: 10.1094/Phyto-78-520

Heiniger, U., and Rigling, D. (1994). Biological control of chestnut blight in Europe. *Annu. Rev. Phytopathol.* 32, 581–599. doi: 10.1146/annurev.py.32.090194.003053

Heydari, A., and Pessarakli, M. (2010). A review on biological control of fungal plant pathogens using microbial antagonists. *J. Biol. Sci.* 10, 273–290. doi: 10.3923/jbs.2010.273.290

Howell, C. R. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Dis.* 87, 4–10. doi: 10.1094/PDIS.2003.87.1.4

Humber, R. A. (1997). "Fungi: Preservation of cultures" in Manual of techniques in insect pathology. ed. L. A. Lacey (Amsterdam: Elsevier), 269–279.

Hyde, K., de Silva, N., Abeywickrama, P., Phookamsak, R., Jeewon, R., Jayawardena, R., et al. (2020). AJOM new records and collections of fungi. *Asian J. Mycol* 3, 1–100. doi: 10.5943/ajom/3/1/3

Kehr, R. D. (1992). *Pezicula canker of Quercus rubra L.*, caused by *Pezicula cinnamomea* (DC.) sacc. *Forest Pathol* 22, 29–40. doi: 10.1111/j.1439-0329.1992.tb01429.x

Kelnarová, I., Černý, K., Zahradník, D., and Koukol, O. (2017). Widespread infection of *Cryptostroma corticale* in asymptomatic *Acer pseudoplatanus* as a risk for urban plantations. *For. Pathol.* 47:e12344. doi: 10.1111/efp.12344

Kenis, M., Hurley, B. P., Colombari, F., Lawson, S., Sun, J. H., Wilcken, C., et al. (2019). Guide to the classical biological control of insect pests in planted and natural forests. *FAO forestry paper* 182. Rome: FAO.

Koukol, O., Kelnarová, I., and Černý, K. (2014). Recent observations of sooty bark disease of sycamore maple in Prague (Czech Republic) and the phylogenetic placement of *Cryptostroma corticale*. *For. Pathol.* 45, 21–27. doi: 10.1111/efp.12129

Kowalski, T., and Kehr, R. (1992). Endophytic fungal colonization of branch bases in several forest tree species. *Sydowia* 44, 137–168.

Langer, G. J. (1994). Die Gattung Botryobasidium DONK (Corticiaceae, Basidiomycetes). Berlin: J. Cramer.

Langer, G. J., and Bußkamp, J. (2021). Fungi associated with Woody tissues of European beech and their impact on tree health. *Front. Virol.* 12:702467. doi: 10.3389/fmicb.2021.702467

Langer, G. J., Bußkamp, J., Terhonen, E., and Blumenstein, K. (2021). "Fungi inhabiting woody tree tissues" in Forest microbiology. eds. J. Mataix-Solera and C. Guerrero (New York, NY: Elsevier), 175–205.

Lundborg, A., and Unestam, T. (1980). Antagonism against fomes annosus. Comparison between different test methods in vitro and in vivo. *Mycopathologia* 70, 107–115. doi: 10.1007/BF00443076

Mapperson, R. R., Kotiw, M., Davis, R. A., and Dearnaley, J. D. W. (2014). The diversity and antimicrobial activity of *Preussia* sp. endophytes isolated from Australian dry rainforests. *Curr. Microbiol.* 68, 30–37. doi: 10.1007/s00284-013-0415-5

Marx, D. H., and Daniel, W. J. (1976). Maintaining cultures of ectomycorrhizal and plant pathogenic fungi in sterile water cold storage. *Can. J. Microbiol.* 22, 338–341. doi: 10.1139/m76-051

Massimo, N. C., Nandi Devan, M. M., Arendt, K. R., Wilch, M. H., Riddle, J. M., Furr, S. H., et al. (2015). Fungal endophytes in aboveground tissues of desert plants: infrequent in culture, but highly diverse and distinctive symbionts. *Microb. Ecol.* 70, 61–76. doi: 10.1007/s00248-014-0563-6

McMullin, D. R., Green, B. D., Prince, N. C., Tanney, J. B., and Miller, J. D. (2017). Natural products of *Picea* endophytes from the Acadian Forest. *J. Nat. Prod.* 80, 1475–1483. doi: 10.1021/acs.jnatprod.6b01157

Moreau, C., and Moreau, M. (1951). La, Suie' des Sycamores a Paris. *Bullet. Soc. Mycol.* 67, 404–418.

Motlagh, M. R. S., and Usefipoor, P. (2016). Identification of non-pathogenic fungi of rice and the evaluation of their effect on biological control of *Pyricularia grisea*, the causal agent of rice blast disease *in vitro*. Hortorum Cultus: Acta Scientiarum Polonorum.

Muller, E., Dvořák, M., Marçais, B., Caeiro, E., Clot, B., Desprez-Loustau, M.-L., et al. (2023). Conditions of emergence of the sooty bark disease and aerobiology of *Cryptostroma corticale* in Europe. *NB* 84, 319–347. doi: 10.3897/neobiota.84.90549

Noble, H. M., Langley, D., Sidebottom, P. J., Lane, S. J., and Fisher, P. J. (1991). An echinocandin from an endophytic *Cryptosporiopsis* sp. and *Pezicula* sp. in *Pinus sylvestris* and *Fagus sylvatica*. *Mycol. Res.* 95, 1439–1440. doi: 10.1016/S0953-7562(09)80401-2

Oliva, J., Ridley, M., Redondo, M. A., and Caballol, M. (2021). Competitive exclusion amongst endophytes determines shoot blight severity on pine. *Funct. Ecol.* 35, 239–254. doi: 10.1111/1365-2435.13692

Oliveira Longa, C. M., Vai, N., and Maresi, G. (2016). *Cryptostroma corticale* in the northern Apennines (Italy). *Phytopathol. Mediterr.* 55, 136–138. doi: 10.14601/Phytopathol_Mediterr-17164

Perlatti, B., Lan, N., Xiang, M., Earp, C. E., Spraker, J. E., Harvey, C. J. B., et al. (2021). Anti-cryptococcal activity of preussolides a and B, phosphoethanolamine-substituted 24-membered macrolides, and leptosin C from coprophilous isolates of *Preussia typharum*. *J. Ind. Microbiol. Biotechnol.* 48:22. doi: 10.1093/jimb/kuab022

Peters, L. P., Prado, L. S., Silva, F. I. N., Souza, F. S. C., and Carvalho, C. M. (2020). Selection of endophytes as antagonists of *Colletotrichum gloeosporioides* in açaí palm. *Biol. Control* 150:104350. doi: 10.1016/j.biocontrol.2020.104350

Peterson, R. A., Bradner, J. R., Roberts, T. H., and Nevalainen, K. M. H. (2009). Fungi from koala (*Phascolarctos cinereus*) faeces exhibit a broad range of enzyme activities against recalcitrant substrates. *Lett. Appl. Microbiol.* 48, 218–225. doi: 10.1111/j.1472-765X.2008.02513.x

Plate, H. P., and Schneider, R. (1965). Ein Fall von asthmaartiger Allergie, verursacht durch den Pilz *Cryptostroma corticale*. *Nachrichtenblatt des deutschen Pflanzenschutzdienstes* 17, 100–101.

Porras-Alfaro, A., Raghavan, S., Garcia, M., Sinsabaugh, R. L., Natvig, D. O., and Lowrey, T. K. (2014). Endophytic fungal symbionts associated with gypsophilous plants. *Botany* 92, 295–301. doi: 10.1139/cjb-2013-0178

Pratt, J. E., Gibbs, J. N., and Webber, J. F. (1999). Registration of *Phlebiopsis gigantea* as a Forest Biocontrol agent in the UK: recent experience. *Biocontrol Sci. Tech.* 9, 113–118. doi: 10.1080/09583159929974

Prospero, S., Botella, L., Santini, A., and Robin, C. (2021). Biological control of emerging forest diseases: how can we move from dreams to reality? *For. Ecol. Manag.* 496:119377. doi: 10.1016/j.foreco.2021.119377

R Core Team. (2019). R Core team (2019). *European Environment Agency*. Available at: https://www.eea.europa.eu/data-and-maps/indicators/oxygen-consuming-substances-in-rivers/r-development-core-team-2006 (Accessed March 18, 2020).

Rai, J. N., and Tewari, J. P. (1963). On some isolates of the genus *Preussia* Fuckel from Indian soils. *Proc. Indian Acad. Sci.* 57, 45–55. doi: 10.1007/BF03053881

Rayner, A. D. M., Griffith, G. S., and Wildman, H. G. (1994). Induction of metabolic and morphogenetic changes during mycelial interactions among species of higher fungi. *Biochem. Soc. Trans.* 22, 389–394. doi: 10.1042/bst0220389

Raziq, F., and Fox, R. T. V. (2003). Comparisons Between the *In Vitro* and *In Vivo* Efficacies of Potential Fungal Antagonists of *Armillaria mellea*. *Biol. Agric. Hortic.* 21, 263–276. doi: 10.1080/01448765.2003.9755269

Rigerte, L., Blumenstein, K., and Terhonen, E. (2019). New R-based methodology to optimize the identification of root endophytes against *Heterobasidion parviporum*. *Microorganisms* 7:102. doi: 10.3390/microorganisms7040102

Rigling, D., and Prospero, S. (2018). *Cryphonectria parasitica*, the causal agent of chestnut blight: invasion history, population biology and disease control. *Mol. Plant Pathol.* 19, 7–20. doi: 10.1111/mpp.12542

Rishbeth, J. (1963). Stump protection against *Fomes annosus*. *Ann. Appl. Biol.* 52, 63–77. doi: 10.1111/j.1744-7348.1963.tb03728.x

Rodriguez, R. J., and Redman, R. S. (1997). Fungal life-styles and ecosystem dynamics: biological aspects of plant pathogens, plant endophytes and saprophytes. *Adv. Bot. Res.* 24, 169–193. doi: 10.1016/S0065-2296(08)60073-7

Rossman, A. Y., Allen, W. C., Castlebury, L., Seifert, K., and Verkley, G. (2018). (2609—2613) proposals to conserve Amorphotheca resinae against *Cladosporium avellaneum*, ditiola mucida (holwaya mucida) against *Acrospermum caliciiforme* (crinula caliciiformis), lophodermium seditiosum against *Leptostroma austriacum*, pezicula sporulosa against *Gloeosporium longisporum* (cryptosporiopsis longispora), and *Tapesia yallundae* (oculimacula yallundae) against Cercosporella herpotrichioides (pseudocercosporella herpotrichioides) (Ascomycota: leotiomycetes). *Taxon* 67, 636–638. doi: 10.12705/673.18

Schlößer, R., Bien, S., Langer, G. J., and Langer, E. J. (2023). Fungi associated with woody tissues of *Acer pseudoplatanus* in forest stands with different health status concerning sooty bark disease (*Cryptostroma corticale*). *Mycol Progress* 22:13. doi: 10.1007/s11557-022-01861-6

Schuldt, B., Buras, A., Arend, M., Vitasse, Y., Beierkuhnlein, C., Damm, A., et al. (2020). A first assessment of the impact of the extreme 2018 summer drought on central European forests. *Basic Appl. Ecol.* 45, 86–103. doi: 10.1016/j.baae.2020.04.003

Schulz, B., Sucker, J., Aust, H. J., Krohn, K., Ludewig, K., Jones, P. G., et al. (1995). Biologically active secondary metabolites of endophytic *Pezicula* species. *Mycol. Res.* 99, 1007–1015. doi: 10.1016/S0953-7562(09)80766-1

Schumacher, J., Leonhard, S., Wulf, A., and Heydeck, P. (2006). Bemerkenswerte Vitalitätsschwächung und Holzzersetzung an Rot-Buchen (*Fagus sylvatica*) durch den weitgehend unbekannten Schlauchpilz *Hypoxylon cohaerens*. *Gesunde Pflanzen* 58, 225–230. doi: 10.1007/s10343-006-0134-y

Shoresh, M., Harman, G. E., and Mastouri, F. (2010). Induced systemic resistance and plant responses to fungal Biocontrol agents. *Annu. Rev. Phytopathol.* 48, 21–43. doi: 10.1146/annurev-phyto-073009-114450

Sieber, T. N. (2007). Endophytic fungi in forest trees: are they mutualists? *Fungal Biol. Rev.* 21, 75–89. doi: 10.1016/j.fbr.2007.05.004

Sinclair, W. A., and Lyon, H. H. (2005). Diseases of trees and shrubs. *2nd* Edn. Ithaca, NY: Cornell University Press, 169–193.

Šnajdr, J., Dobiášová, P., Větrovský, T., Valášková, V., Alawi, A., Boddy, L., et al. (2011). Saprotrophic basidiomycete mycelia and their interspecific interactions affect the spatial distribution of extracellular enzymes in soil. *FEMS Microbiol. Ecol.* 78, 80–90. doi: 10.1111/j.1574-6941.2011.01123.x

Song, Z.-K., Zhu, A.-H., Liu, Z.-D., Qu, Z., Li, Y., and Ma, H.-X. (2022). Three new species of *Hypoxylon* (*Xylariales*, Ascomycota) on a multigene phylogeny from Medog in Southwest China. *JoF* 8:500. doi: 10.3390/jof8050500

Sonnenbichler, J., Dietrich, J., and Peipp, H. (1994). Secondary fungal metabolites and their biological activities, V. Investigations concerning the induction of the biosynthesis of toxic secondary metabolites in Basidiomycetes. *Biol. Chem. Hoppe Seyler* 375, 71–80. doi: 10.1515/bchm3.1994.375.1.71

Stevens, R., Reyes, G., and Kanavillil, N. (2020). Examining the impact of winter and spring soil temperatures on the growth of *Hypholoma fasciculare*, a potential Biocontrol agent against *Armillaria ostoyae*, in pine plantations. *Front. For. Glob. Change* 3:598527. doi: 10.3389/ffgc.2020.598527

Taylor, A. G., and Harman, G. E. (1990). Concepts and Technologies of Selected Seed Treatments. *Annu. Rev. Phytopathol.* 28, 321–339. doi: 10.1146/annurev.py.28.090190.001541

Thompson, T. A., Thorn, R. G., and Smith, K. T. (2012). *Hypholoma lateritium* isolated from coarse woody debris, the forest floor, and mineral soil in a deciduous forest in New Hampshire. *Botany* 90, 457–464. doi: 10.1139/b2012-011

Thomson, B. D., Malajczuk, N., Grove, T. S., and Hardy, G. E. (1993). Improving the colonization capacity and effectiveness of ectomycorrhizal fungal cultures by association with a host plant and re-isolation. *Mycol. Res.* 97, 839–844. doi: 10.1016/S0953-7562(09)81159-3

Towey, J. W., Sweany, H. C., and Huron, W. H. (1932). Severe bronchial asthma apparently due to fungus spores found in maple bark. *JAMA* 99, 453–459. doi: 10.1001/jama.1932.02740580021005

Wang, Q., and Xu, L. (2012). Beauvericin, a bioactive compound produced by Fungi: a short review. *Molecules* 17, 2367–2377. doi: 10.3390/molecules17032367

Weber, H. A., and Gloer, J. B. (1988). Interference competition among natural fungal competitors: an antifungal metabolite from the coprophilous fungus *Preussia fleischhakii*. *J. Nat. Prod.* 51, 879–883. doi: 10.1021/np50059a011

Weber, H. A., and Gloer, J. B. (1991). The Preussomerins: novel antifungal metabolites from the coprophilous fungus *Preussia isomera* Cain. *J. Org. Chem.* 56, 4355–4360. doi: 10.1021/jo00014a007

Wendt, L., Sir, E. B., Kuhnert, E., Heitkämper, S., Lambert, C., Hladki, A. I., et al. (2018). Resurrection and emendation of the Hypoxylaceae, recognised from a multigene phylogeny of the Xylariales. *Mycol Progress* 17, 115–154. doi: 10.1007/s11557-017-1311-3

Whipps, J. M. (1987). Effect of media on growth and interactions between a range of soil-borne glasshouse pathogens and antagonistic fungi. *New Phytol.* 107, 127–142. doi: 10.1111/j.1469-8137.1987.tb04887.x

Whipps, J. M., and Gerlagh, M. (1992). Biology of *Coniothyrium minitans* and its potential for use in disease biocontrol. *Mycol. Res.* 96, 897–907. doi: 10.1016/S0953-7562(09)80588-1

Woods, C. M., Woodward, S., and Redfern, D. B. (2005). In vitro interactions in artificial and wood-based media between fungi colonizing stumps of Sitka spruce. *For. Pathol.* 35, 213–229. doi: 10.1111/j.1439-0329.2005.00403.x

Woodward, S., and Boddy, L. (2008). "Chapter 7 interactions between saprotrophic fungi" in Ecology of saprotrophic Basidiomycetes. eds. L. Boddy and J. Frankland (New York, NY: Elsevier), 125–141.

Young, C. W. T. (1978). Sooty bark disease of sycamore. London: Stationery Office Books, Department of Environment.

Yuan, Z.-L., Rao, L.-B., Chen, Y.-C., Zhang, C.-L., and Wu, Y.-G. (2011). From pattern to process: species and functional diversity in fungal endophytes of *Abies beshanzuensis*. *Fungal Biol.* 115, 197–213. doi: 10.1016/j.funbio.2010.11.002

Yuen, T. K., Hyde, K. D., and Hodgkiss, I. J. (1999). Interspecific interactions among tropical and subtropical freshwater Fungi. *Microb. Ecol.* 37, 257–262. doi: 10.1007/s002489900151

4 General Discussion

4.1 Mycobiome of trees and endophytes of trees

Prior to the study presented in chapter 2 there had been no recent comprehensive works investigating the fungal community in *Acer pseudoplatanus*, particularly in woody tissue. Since endophytes are becoming continuously more important in research regarding forest trees, the study provides an overview of the present mycobiome in *A. pseudoplatanus* woody tissue in Germany. Future explorations of the same tissue in different locations could help to form a more complete picture of the full mycobiome found in woody tissue of *A. pseudoplatanus*. In comparison to other studies, the amount of isolated morphotypes in the presented study is notably higher (Butin and Kowalski 1986; Kowalski and Kehr 1992; Unterseher et al. 2005; Brglez et al. 2020). One main difference between the cited works and the presented one is the difference in sampling size as well as the number of sites sampled, which can explain the difference in isolated morphotypes. Sampling procedure and identification also vary greatly. Cultures in the presented study (Schlößer et al. 2023) were grouped into morphotypes and later genetically determined. In comparison Kowalski and Kehr (1992) isolated from living parts of branch bases and determined cultures solely on morphological basis. None the less, the amount of tissue samples in the presented study is still a relatively small portion in regard to the entire body of wood. It is likely that if more samples were taken, even more taxa would have been discovered. The observed differences regarding the fungal composition between individual trees were large, likewise between the different sites. Another sampling site might have added many additional morphotypes, as it is known that the amount of fungal species detected increases with the amount of sampled material (*e.g.* Peters et al. 2023). Differences in the composition of the fungal community in general have also been noted in different types of tissue (Gennaro et al. 2003) as well as between trees of different ages (Halley et al. 1994; Maherali and Klironomos 2007). It is also known that the mycobiome of trees can differ strongly between different forest stands and even individual trees (*e.g.* Hoffman and Arnold 2008; Franić et al. 2023).

In Chapter 2, several fungi were isolated from discoloured tissue though the agent causing the symptoms was not identified. *Biscogniauxia nummularia* (Bull.) Kuntze, for example, which is a known pathogen on beech (Granata and Sidoti 2004), was isolated from the discoloured material. *Diaporthe* sp. and a *Neonectria* sp. were also isolated from discoloured material. Both of these genera are known to host pathogenic species (Gomes et al. 2013; Gómez-Cortecero et al. 2016; Karadžić et al. 2020; González et al. 2021; Hilário et al. 2021).

The isolated fungal community being dominated by the *Ascomycota* (Schlößer et al. 2023) is congruent with the results of other studies conducted on this topic (Petrini and Fisher 1988; Kowalski and Kehr 1992; Peršoh et al. 2010; Martínez-Álvarez et al. 2012; Sanz-Ros et al. 2015). Through recent efforts of high throughput sequencing it was discovered though, that the amount of fungal species present in the wood, specifically *Basidiomycota*, is largely underestimated (Rungjindamai and Jones 2024 and literature cited herein). Exclusively using culture-based methods of isolations will represent only a portion of the present fungi. Furthermore, *Basidiomycota* often exhibit a slower growth compared to *e.g. Ascomycota* (Oses et al. 2008) and are thus easily overgrown. *Basidiomycota* commonly grow better on selective nutrient media and at lower temperatures (Rungjindamai and Jones 2024). In the study presented in chapter 2, only one type of nutrient media was used for isolation, which could partially explain the low amount of *Basidiomycota* found, but also other species. Thus, the amount of *Basidiomycota*, as well as the total amount of fungi present in *Acer* trees is most likely higher and more diverse than presented in this study (Guo et al. 2001; Allen et al. 2003; Unterseher 2007; Muggia et al. 2017). By using a genetically-based approach like High Throughput Sequencing (HTS) or Next-Generation Sequencing (NGS) a larger diversity of species could likely have been discovered (Allen et al. 2003; Blumenstein et al. 2021; Yasanthika et al. 2022). On the downside, using solely a genetically based approach can lead to missing species which could have been detected using a culture-based approach, as shown by Blumenstein et al. (2021), who did not detect several species of the *Xylariales*, found by the culture-based analysis, through HTS. Thus, it appears advisable to use a combined approach in order to get the results with the best resolution.

Since the determination of species in this study was primarily based on ITS (internally transcribed sequence) sequences, it is possible that with other markers the isolates not determined to species level could have been determined further. ITS sequences, depending on the species-complex or the genus, can have a high intraspecific variation (Lacap et al. 2003). Thus, the ITS sequence is not sufficient to determine an isolate to species level (Lacap et al. 2003; Hoffman and Arnold 2008). For this reason, the Actin and β-tubulin region were used in in addition to the ITS region, in order to be able to be more specific regarding the genus *Neonectria*.

Given the existence of the endophytic stage of *C. corticale*, which had long been presumed (Gibbs 1998; Sieber 2007), it is likely that the distribution range of the fungus is much larger than assumed until now based on recorded outbreaks. As previously stated, the periodical outbreaks of the disease often coincide with above average summer temperatures and subsequent draught causing stress in the plant (Gregory and Waller 1951; Dickenson 1980), suggesting an already established presence of the fungus in the plant. Furthermore, Muller et al. (2023) showed that spores of *C. corticale* can be detected at a distance of at least 310 km from the closest known site of infection, emphasizing the potential spread of this fungus. Given its presence on the European mainland since the 1950s (Moreau and Moreau 1951) the author hypothesizes that a large part of *A. pseudoplatanus* populations/ stands in central Europe may already be colonized by *C. corticale*.

No outbreaks of SBD had been reported in the forests of Schleswig-Holstein during the study period. Thus, in chapter 2 the sampling site in Nehmten was chosen out of interest to see whether *C. corticale* was possibly present in Schleswig-Holstein in an endophytic or latent stage. During the analysis *C. corticale* was indeed isolated from the Nehmten samples, proving its presence even though no outbreaks of SBD in forests had yet been reported. A newly reported outbreak of SBD from forests in Schleswig-Holstein is located on the island of Fehmarn, close to Denmark in the north of Germany (Nordwestdeutsche Forstliche Versuchsanstalt and Hessisches Ministerium für Landwirtschaft und Umwelt, Weinbau, Forsten, Jagd und Heimat 2024). This report emphasizes the wide distribution of the causal agent, and its continuous emergence as well as its potential to further spread to the north.

Several fungi including *C. corticale*, can switch between different lifestyles (Ragazzi et al. 2003; Hyde and Soytong 2008; Sanz-Ros et al. 2015). Consequently, the classification of lifestyle for the fungi isolated in Schlößer et al. (2023) is not straightforward and was not investigated in the presented study. For this reason, we refer to fungi associated with living woody tissues rather than endophytes, as we have not demonstrated that each fungus was isolated in its endophytic stage. Most of the fungi were only isolated once, making such a determination challenging. As observed especially during extreme weather events in summer, the impact of the climatic conditions can lead to a switch of lifestyle in several fungi, *e.g.* causing them to become pathogenic (Sieber 2007; Langer et al. 2021; Singh et al. 2023).

4.2 Biological control and the use of endophytes in biological control

From all the different mechanisms which can be at play in antagonistic interactions, the study presented in chapter 3 (Schlößer et al. 2025) only takes competition in consideration, since no further analyses were carried out regarding *e.g.* organic compounds. Even though only competition was considered in the presented study, according to Woodward and Boddy (2008) and Boddy (2016) antagonistic interactions at a distance are commonly caused by organic compounds being emitted by one or both fungi. The use of fungi already known to be associated with the host plant, not necessarily already present, by means of targeted biological control could be beneficial. It has to be considered though that the use of non-coevolved endophytes as BCA could alter or negatively impact the mycobiome of the host tree (Rabiey et al. 2019; Prospero et al. 2021). This can for example happen through antibiosis of broad-spectrum BCAs (Prospero et al. 2021).

The tests described in chapter 3 showed a more prominent antagonism after the potential antagonist had been transferred into the dual-culture one week prior to *C. corticale*. This suggests that, when given time, the potential antagonists can emit a higher amount or more potent organic compounds. As Test 4 showed, many of the sampled hyphal tips were still viable. This leads to the assumption, that most of the interactions resulting in antagonism in this study exhibit a fungistatic rather than a fungicidal effect. The use of organic compounds, specifically secondary metabolites, for biological control appears to be a promising alternative to the use of chemical control or living organisms. Their production is strongly dependent on biotic as well as abiotic factors such as temperature, pH, and humidity (Nielsen et al. 2004; Keller et al. 2005; Qaderi et al. 2023), and the given culture conditions (Blom et al. 2011), making their production by an organism in nature unpredictable. Several secondary metabolites are known to have antifungal, antibacterial, or antimicrobial effects, such as alternaric acid (Brian et al. 1949), cryptosporiopsin (Schulz et al. 1995), α-muurolene (Baptista et al. 2021), and beauvericin (Keswani et al. 2019). In other reported cases a combination of beauvericin and ketoconazole, for example, was found to cause mortality of *Candida parapsilosis* (Ashford) Langeron & Talice by Wang and Xu (2012). For two of the proposed potential antagonists the production of antimicrobial/antifungal compounds is known. Namely, *Hypholoma fasciculare* (Huds.) P. Kumm. and *Pezicula sporulosa* Verkley (McMullin et al. 2017; Baptista et al. 2021). The use of an organic compound could also ensure the effectiveness of the control against different strains of the pathogen. Identifying a specific compound could provide a more reliable approach regarding a potential control against *C. corticale*. Given that *H. fasciculare* is known as a saprophyte causing rot in beech (Folman et al. 2008; Šnajdr et al. 2011) and *Jackrogersella cohaerens* (Pers.) L. Wendt, Kuhnert & M. Stadler is a known saprophyte on beech which can also cause disease in beech trees (Sinclair and Lyon 2005; Schumacher et al. 2006) they are not ideal candidates for biological control agents. Yet, identifying organic compounds involved in the antagonistic reactions could forego the problematic of potential negative effects of the fungus on the host plant. An approach based on Integrated Pest Management (IPM), *i.e.* using sustainable and biological means of control, appears the most beneficial for both the environment and the subsequent effects on animals and humans.

To ensure a safe and beneficial use of the proposed potential antagonists it would be necessary to test the harmlessness of the selected fungal strains against *A. pseudoplatanus in vivo*. Alternatively, the abovementioned organic compounds could be explored further as an option for means of biological control. This could not only be a beneficial solution in regard to damage to the plant but also in ensuring the needed compounds and factors for control are present. The search for, and identification of, an effective universal biological control agent for *C. corticale* is a delicate matter with many factors to be considered. Even with the results obtained in the study presented in chapter 3 there are still many obstacles to overcome until a potential control could be used commercially. Two important factors to consider would be the mode of application as well as the time and effort needed to apply such a BCA. Against foliar pathogens like *Botrytis subtilis* an aqueous spray is applied on the leaves of nut or fruit trees (Rabiey et al. 2019). Applying a biological control to the wood body of a tree is more challenging, though, since corky bark layers may prevent successful direct application of spore suspensions etc. to the outer surface of the tree. In the case of *Cryphonectria parasitica*, the biological control by application of a strain of the pathogen infected with a hyperparasite, is applied annually by small injections directly into the trunk of *Castanea* spp. trees (Heiniger and Rigling 1994; Rigling and Prospero 2018). A similar method is used for the treatment of *Phytophthora cinnamomi* Rands. with strains of *Bacillus* sp. in avocado trees to reduce the disease severity (Darvas and Bezuidenhout 1987), making this a possible application method for single trees in the presented pathosystem.

It should not be dismissed that with the continuously growing global trade harmful organisms can be introduced to non-native regions as well as regions they had not been previously present in (Westphal et al. 2008; Santini et al. 2013). Many known pathogens have continuously emerged in new regions in the past decades. These include for example *Ceratocystis platani* which was introduced, *e.g.* to Greece and Turkey (Ocasio-Morales et al. 2007; Lehtijärvi et al. 2018), *Hymenoscyphus fraxineus* which was introduced to Europe and first detected in Poland (Przybył 2002), and *Xylella fastidiosa* Wells introduced to several regions worldwide including Italy, Brazil and the western United States (Sicard et al. 2018). SBD is currently emerging in several new regions in North America and Europe, most recently in Washington State, USA, British Columbia, Canada and Russia (Brooks et al. 2023; Gninenko et al. 2024; Tanney et al. 2024). This is also the case in other regions, such as the Apennine region in Italy (Schlößer et al. 2024). This emphasized the importance of being able to protect trees against an outbreak of the SBD.

4.3 Outlook on the future of *Acer pseudoplatanus* in regard to SBD and impact of SBD in European forests

Interestingly, *C. corticale* was the most abundant species found in Schlößer et al. (2023) among fungi isolated from visually healthy trees. This leads to assume that the fungus has a much bigger range of distribution than monitored by outbreaks of SBD. Given the periodic reoccurrence of the disease following years with above average summer temperatures it is likely that the disease will stay present in the face of climate change. Regarding the intensity and significance of the disease no predictions can be made as this also appears to be highly dependent on climatic conditions including drought and high summer temperatures. Periods of extreme drought like experienced in the years 2018-2020 (DWD 2018, 2019, 2020) appear to amplify disease outbreaks (Young 1978; Dickenson and Wheeler 1981). One of the likely reasons is the exhaustion of the soil water storage (Dickenson 1980; Dickenson and Wheeler 1981). Through experiencing drought stress the trees get weakened and are unable to activate their defences quickly and effectively. Outbreaks of the SBD have been reported from all over Europe (Moreau and Moreau 1951; Gregory and Waller 1951; Plate and Schneider 1965; Abbey and Stretton 1985; Cech 2004; Engesser et al. 2004; Metzler 2006; Robeck et al. 2008; Langer et al. 2013; Bencheva 2014; Koukol et al. 2014; Oliveira Longa et al. 2016; Kelnarová et al. 2017; Muller et al. 2023). As it appears possible that a large amount of *Acer pseudoplatanus* populations and stands in Europe are already colonised by *Cryptostroma corticale*, future outbreaks at differing scales throughout Europe are likely to occur.

Regarding the future of *A. pseudoplatanus* in forests it is important to plant the species on suitable site, meeting the needs of water and nutrient availability. Losses will probably still have to be accepted as even the best forest stand will potentially not be able to compensate extreme climatic conditions. Just because *C. corticale* is present an outbreak is not necessarily inevitable. Sanitary cuts of diseased plants are optional. More importantly, new infections through fresh wounds caused during measures should be avoided.

In regard to the presence of *A. pseudoplatanus* in urban areas, trees should be controlled regularly for symptoms, especially early symptoms like wilting. Water availability should be given and possibly supplemented during extreme drought periods in order to avoid drought stress in the tree. If an infection is detected, trees should be cut as the spores can cause an allergic reaction in humans. Furthermore, a visible sporulation of *C. corticale* also implies rot being present in the trunk, *i.e.* also the stability of the tree might be compromised and cause a potential risk to the road safety.

5 Conclusion

The insight into the mycobiome of living woody tissue of *A. pseudoplatanus* obtained and the confirmation of the endophytic stage of *C. corticale* provide a basis further investigation into the mycobiome as well as the biological control of the SBD. The study presented in chapter 3 constitutes the first published attempt at finding a potential BCA effective against *Cryptostroma corticale*. The studies comprising this thesis show the possibility of using fungi already associated with a host tree as a biological control agent. Several other studies have applied the same approach in different pathosystems. The most obvious benefits of using fungi already associated with the mycobiome of the host plant are a) not having to introduce an unknown organism and b) avoiding the use of chemical control agents. A proactive approach to plant health is needed through IPM not only to protect forest from already present threats but also against potential threats which might arise in the future. Regarding the current prospects of climate change it is important to have means of control available since it is likely that large scale outbreaks of *C. corticale* will become more frequent. Establishing an effective control against *C. corticale* is not only relevant in regard to forest ecosystems suffering an economic and ecological loss but also for urban forests, predominantly in central Europe, where *A. pseudoplatanus* is a commonly used species in both environments.

References

Abbey SD (1978) The morphology and physiology of *Cryptostroma corticale*. Thesis, Loughborough University.

Abbey SD, Stretton RJ (1985) Some environmental factors affecting *Cryptostroma corticale*. Microbios 44:157–167.

Abbott RJ, Bevercombe GP, Rayner ADM (1977) Sooty bark disease of sycamore and the grey squirrel. Transactions of the British Mycological Society 69:507–508. https://doi.org/10.1016/S0007-1536(77)80095-8

Adeleke BS, Babalola OO (2021) The plant endosphere-hidden treasures: a review of fungal endophytes. Biotechnology and Genetic Engineering Reviews 37:154–177. https://doi.org/10.1080/02648725.2021.1991714

Allen TR, Millar T, Berch SM, Berbee ML (2003) Culturing and direct DNA extraction find different fungi from the same ericoid mycorrhizal roots. New Phytologist 160:255–272. https://doi.org/10.1046/j.1469-8137.2003.00885.x

Arnold AE, Lutzoni F (2007) Diversity and Host Range of Foliar Fungal Endophytes: Are Tropical Leaves Biodiversity Hotspots? Ecology 88:541–549.

Aswani R, Roshmi T, Radhakrishnan EK (2022) Induction of plant defense response by endophytic microorganisms. In: Biocontrol Mechanisms of Endophytic Microorganisms. Academic Press, pp 89–115.

Avila AL de, Häring B, Rheinbay B, et al (2021) Artensteckbriefe 2.0: alternative Baumarten im Klimawandel: eine Stoffsammlung. Forstliche Versuchs- und Forschungsanstalt Baden-Württemberg, Freiburg im Breisgau.

Baptista P, Guedes De Pinho P, Moreira N, et al (2021) *In vitro* interactions between the ectomycorrhizal *Pisolithus tinctorius* and the saprotroph *Hypholoma fasciculare* fungi: morphological aspects and volatile production. Mycology 12:216–229. https://doi.org/10.1080/21501203.2021.1876778

Bencheva S (2014) First report of *Cryptostroma corticale* (Ellis & Everh.) P.H. Greg. & S. Waller on *Acer platanoides* L. in Bulgaria. Silva Balcania 15(2):101–104.

BfN (2025) Gebietsfremde und invasive Arten. In: Bundesamt für Naturschutz. https://www.bfn.de/gebietsfremde-und-invasive-arten

Blom D, Fabbri C, Connor EC, et al (2011) Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions. Environmental Microbiology 13:3047–3058. https://doi.org/10.1111/j.1462-2920.2011.02582.x

Blumenstein K, Bußkamp J, Langer GJ, et al (2021) The Diplodia Tip Blight Pathogen *Sphaeropsis sapinea* Is the Most Common Fungus in Scots Pines' Mycobiome, Irrespective of Health Status—A Case Study from Germany. JoF 7:607. https://doi.org/10.3390/jof7080607

Boddy L (2000) Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Microbiology Ecology 31:185–194. https://doi.org/10.1111/j.1574-6941.2000.tb00683.x

Boddy L (2016) Interactions Between Fungi and Other Microbes. In: The Fungi. Elsevier, pp 337–360. https://doi.org/10.1016/B978-0-12-382034-1.00010-4

Bonello, Gordon, Storer (2001) Systemic induced resistance in Monterey pine. Forest Pathology 31:99–106. https://doi.org/10.1046/j.1439-0329.2001.00230.x

Brenken A-C, Kehr R, Riebesehl J, et al (2024) First report of *Cryptostroma corticale* on *Aesculus hippocastanum* causing sooty bark disease in Germany. J Plant Dis Prot 131:1087–1092. https://doi.org/10.1007/s41348-024-00891-4

Brglez A, Piškur B, Ogris N (2020) *Eutypella parasitica* and Other Frequently Isolated Fungi in Wood of Dead Branches of Young Sycamore Maple (*Acer pseudoplatanus*) in Slovenia. Forests 11:467. https://doi.org/10.3390/f11040467

Brian PW, Curtis PJ, Hemming HG, et al (1949) Alternaric Acid, a Biologically Active Metabolic Product of the Fungus *Alternaria solani*. Nature 164:534

Brooks RK, Omdal D, Brown S, et al (2023) *Cryptostroma corticale*, the causal agent of sooty bark disease of maple, appears widespread in western Washington State, USA. Forest Pathology 53:6. https://doi.org/10.1111/efp.12835

Brosinger F, Schmidt O (2009) Der Bergahorn in Bayern. LWF Wissen Bayerische Landesanstalt für Wald und Forstwirtschaft: Freising 19–23.

Burgdorf N, Blaschke M, Petercord R, et al (2014) *Verticillium*-Welke bei Bergahorn Verbreitung von *Verticillium dahliae* in bayerischen Waldbeständen im Zusammen-hang mit Stammnekrosen bei Bergahorn. LWF Aktuell 102.

Busby PE, Ridout M, Newcombe G (2016) Fungal endophytes: modifiers of plant disease. Plant Mol Biol 90:645–655. https://doi.org/10.1007/s11103-015-0412-0

Bußkamp J, Bien S, Neumann L, et al (2024) Endophytic community in juvenile *Acer pseudoplatanus* and pathogenicity of *Cryptostroma corticale* and other associated fungi under controlled conditions. J Plant Pathol 106:565–577. https://doi.org/10.1007/s42161-023-01575-y

Butin H, Kowalski T (1986) Die natürliche Astreinigung und ihre biologischen Voraussetzungen. European Journal of Forest Pathology 16:129–138. https://doi.org/10.1111/j.1439-0329.1986.tb01053.x

Caarls L, Pieterse CMJ, Van Wees SCM (2015) How salicylic acid takes transcriptional control over jasmonic acid signaling. Front Plant Sci 6:170. https://doi.org/10.3389/fpls.2015.00170

CABI (2022) *Acer pseudoplatanus* (sycamore). CABI Compendium CABI Compendium:2884. https://doi.org/10.1079/cabicompendium.2884

Campanile G, Ruscelli A, Luisi N (2007) Antagonistic activity of endophytic fungi towards *Diplodia corticola* assessed by *in vitro* and *in planta* tests. Eur J Plant Pathol 117:237–246. https://doi.org/10.1007/s10658-006-9089-1

Carnegie AJ, Lidbetter JR, Walker J, et al (2010) *Uredo rangelii*, a taxon in the guava rust complex, newly recorded on Myrtaceae in Australia. Austral Plant Pathol 39:463. https://doi.org/10.1071/AP10102

Carroll G (1988) Fungal Endophytes in Stems and Leaves: From Latent Pathogen to Mutualistic Symbiont. Ecology 69:2–9. https://doi.org/10.2307/1943154

Cech TL (2004) Bermerkenswerte Pilzkrankheiten in 2004. Forstschutz Aktuell 32:31–34.

Chen D, Mubeen B, Hasnain A, et al (2022) Role of Promising Secondary Metabolites to Confer Resistance Against Environmental Stresses in Crop Plants: Current Scenario and Future Perspectives. Front Plant Sci 13:881032. https://doi.org/10.3389/fpls.2022.881032

Chlebicki A (1988) Some ascomycetous fungi or their anamorphs occurring on trees in Poland I. Acta Mycologica 24:77–92.

Conrath U, Beckers GJM, Flors V, et al (2006) Priming: Getting Ready for Battle. MPMI 19:1062–1071. https://doi.org/10.1094/MPMI-19-1062

Conrath U, Thulke O, Katz V, et al (2001) Priming as a Mechanism in Induced Systemic Resistance of Plants. European Journal of Plant Pathology 107:113–119. https://doi.org/10.1023/A:1008768516313

Danielsen S, Jensen DF (1999) Fungal Endophytes from Stalks of Tropical Maize and Grasses: Isolation, Identification, and Screening for Antagonism against *Fusarium verticillioides* in Maize Stalks. Biocontrol Science and Technology 9:545–553. https://doi.org/10.1080/09583159929505

Darvas JM, Bezuidenhout JJ (1987) Control of *Phytophthora* root rot of avocados by trunk injection. South African Avocado Growers' Association Yearbook 91–93.

Davis E, The Maple Society (2021) Systematic Classification of Acer - A Survey of the genus in accordance with The Maple Society Accepted Species Names.

de Bary A (1866) Morphologie und Physiologie der Pilze, Flechten und Myxomyceten. W. Engelmann, Leipzig.

Dickenson S, Wheeler BEJ (1981) Effects of temperature, and water stress in sycamore, on growth of *Cryptostroma corticale*. Transactions of the British Mycological Society 76:181–185. https://doi.org/10.1016/S0007-1536(81)80136-2

Dickenson SJ (1980) Biology of *Cryptostroma corticale* and the sooty bark disease of sycamore. Dissertation, University of Bath.

DWD (2004) Das Wetter in Deutschland in Jahr 2003: Sonnig, warm und trocken - Superjahr mit vielen Wetterrekorden. DWD (Deutscher Wetterdienst), Offenbach.

DWD (2005) Jahresrückblick: Deutschlandwetter im Jahr 2005 Deutsche konnten 2005 reichlich Wärme und Sonne genießen. DWD (Deutscher Wetterdienst), Offenbach.

DWD (2018) Deutschlandwetter im Jahr 2018 - 2018 - ein außergewöhnliches Wetterjahr mit vielen Rekorden. DWD (Deutscher Wetterdienst), Offenbach.

DWD (2019) Deutschlandwetter im Jahr 2019 – Drittwärmstes Jahr seit 1881 – Niederschlagsarm und sonnenscheinreich. DWD (Deutscher Wetterdienst), Offenbach.

DWD (2020) Deutschlandwetter im Jahr 2020 - 2020 bestätigt als zweitwärmstes Jahr den fortschreitenden Klimawandel. DWD (Deutscher Wetterdienst), Offenbach.

Ellis JB, Everhart, BM (1889) New species of hyphomycetes fungi. Journal of Mycology 5:68–72.

Ellis MB, Ellis PJ (1985) Microfungi on land plants: an identification handbook. Croom Helm Ltd., London.

Enderle R, Riebesehl J, Becker P, Kehr R (2020) Rußrindenkrankheit an Ahorn - Biologie, Pathologie und Entsorgung von Schadholz. In: Dujesiefken D (ed) Jahrbuch der Baumpflege 2020, 24th edn. Haymarket Media, Braunschweig, pp 85–100.

Engesser R, Forster B, Meier E, Odermatt O (2004) Forstschutzsituation 2003 in der Schweiz. AFZ - Der Wald 59:385–387

Eschen R, O'Hanlon R, Santini A, et al (2019) Safeguarding global plant health: the rise of sentinels. J Pest Sci 92:29–36. https://doi.org/10.1007/s10340-018-1041-6

EU (2019) EU 2019/2072.

Eyles A, Bonello P, Ganley R, Mohammed C (2010) Induced resistance to pests and pathogens in trees. New Phytologist 185:893–908. https://doi.org/10.1111/j.1469-8137.2009.03127.x

Feng SR (2008) *Acer laurinum*. Flora of China 11:547.

Folman LB, Klein Gunnewiek PJA, Boddy L, De Boer W (2008) Impact of white-rot fungi on numbers and community composition of bacteria colonizing beech wood from forest soil: Impact of white-rot fungi on bacteria colonizing wood. FEMS Microbiology Ecology 63:181–191. https://doi.org/10.1111/j.1574-6941.2007.00425.x

Fourrier C, Antoine S, Piou D, Ioos R (2015) Rapid detection of *Fusarium circinatum* propagules on trapped pine beetles. Forest Pathology 45:324–330. https://doi.org/10.1111/efp.12173

Franić I, Allan E, Prospero S, et al (2023) Climate, host and geography shape insect and fungal communities of trees. Sci Rep 13:11570. https://doi.org/10.1038/s41598-023-36795-w

GALK (2025) GALK - Straßenbaumliste Abfrage vom 07.02.2025 Arbeitskreis Stadtbäume.

Garbelotto M, Popenuck T, Schmidt D, et al (2024) First Report of *Cryptostroma corticale* Causing Sooty Bark Disease in California and First Worldwide Report of Silver Maple as a Host. Plant Disease 108:1395. https://doi.org/10.1094/PDIS-12-23-2734-PDN

Gennaro M, Gonthier P, Nicolotti G (2003) Fungal Endophytic Communities in Healthy and Declining *Quercus robur* L. and *Q. cerris* L. Trees in Northern Italy. J Phytopathol 151:529–534. https://doi.org/10.1046/j.1439-0434.2003.00763.x

Gibbs D, Chen Y (2009) The red list of maples. Botanic Gardens Conservation International, Richmond.

Gibbs JN (1998) Understanding and Controlling diseases of woody plants: a historical perspective. In: Paper No. 3.7.1S. Edinburgh.

Glass NL, Dementhon K (2006) Non-self recognition and programmed cell death in filamentous fungi. Current Opinion in Microbiology 9:553–558. https://doi.org/10.1016/j.mib.2006.09.001

Gninenko YuI, Chilakhsaeva EA, Seraya LG, et al (2024) First Report of *Cryptostroma corticale*, a Causative Agent of the Sooty Bark Disease of Maples, in Russia. Russ J Biol Invasions 15:26–31. https://doi.org/10.1134/S2075111724010041

Gomes RR, Glienke C, Videira SIR, et al (2013) *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia 31:1–41. https://doi.org/10.3767/003158513X666844

Gómez-Cortecero A, Saville RJ, Scheper RWA, et al (2016) Variation in Host and Pathogen in the *Neonectria* / *Malus* Interaction; toward an Understanding of the Genetic Basis of Resistance to European Canker. Front Plant Sci 7:. https://doi.org/10.3389/fpls.2016.01365

González M, Reynolds C, Forster J, et al (2021) Incidence of the emerging pathogen *Neonectria neomacrospora* on *Abies* taxa in the National Arboreta in England (UK). Forest Ecology and Management 492:119207. https://doi.org/10.1016/j.foreco.2021.119207

Granata G, Sidoti A (2004) *Biscogniauxia nummularia*: pathogenic agent of a beech decline. Forest Pathology 34:363–367. https://doi.org/10.1111/j.1439-0329.2004.00377.x

Gregory PH, Waller S (1951) *Cryptostroma corticale* and sooty bark disease of sycamore (*Acer pseudoplatanus*). Transactions of the British Mycological Society 34:579–597. https://doi.org/10.1016/S0007-1536(51)80043-3

Gross A, Holdenrieder O, Pautasso M, et al (2014) *Hymenoscyphus pseudoalbidus* the causal agent of European ash dieback. Molecular Plant Pathology 15:5–21. https://doi.org/10.1111/mpp.12073

Guo LD, Hyde KD, Liew ECY (2001) Detection and taxonomic placement of endophytic fungi within frond tissues of *Livistona chinensis* based on rDNA sequences. Molecular Phylogenetics and Evolution 20:1–13. https://doi.org/10.1006/mpev.2001.0942

Halley JM, Comins HN, Lawton JH, Hassell MP (1994) Competition, Succession and Pattern in Fungal Communities: Towards a Cellular Automaton Model. Oikos 70:435–442. https://doi.org/10.2307/3545783

Hamelin RC, Roe AD (2020) Genomic biosurveillance of forest invasive alien enemies: A story written in code. Evolutionary Applications 13:95–115. https://doi.org/10.1111/eva.12853

Hammerschmidt R, Kuć J (eds) (1995) Induced Resistance to Disease in Plants. Springer Netherlands, Dordrecht.

Hawksworth DL, Rossman AY (1997) Where Are All the Undescribed Fungi? Phytopathology® 87:888–891. https://doi.org/10.1094/PHYTO.1997.87.9.888

Hein S (2004) Zur Steuerung von Astreinigung und Dickenwachstum bei Esche (*Fraxinus excelsior* L.) und Bergahorn (*Acer pseudoplatanus* L.). Fakultät für Forst und Umweltwissenschaften, Albert-Ludwigs-Universität Freiburg i. Brsg.

Heiniger U, Rigling D (1994) Biological Control of Chestnut Blight in Europe. Annu Rev Phytopathol 32:581–599. https://doi.org/10.1146/annurev.py.32.090194.003053

Herre EA, van Bael SA, Maynard Z, et al (2005) Tropical plants as chimera: some implications of foliar endophytic fungi for the study of host-plant defence, physiology and genetics. In: Biotic interactions in the Tropics: their role in the maintenance of species diversity. Cambridge university press, Cambridge, pp 226–240.

Heydari A, Pessarakli M (2010) A Review on Biological Control of Fungal Plant Pathogens Using Microbial Antagonists. J of Biological Sciences 10:273–290. https://doi.org/10.3923/jbs.2010.273.290

Hilário S, Santos L, Alves A (2021) Diversity and Pathogenicity of *Diaporthe* Species Revealed from a Survey of Blueberry Orchards in Portugal. Agriculture 11:. https://doi.org/10.3390/agriculture11121271

Hoffman MT, Arnold AE (2008) Geographic locality and host identity shape fungal endophyte communities in cupressaceous trees. Mycological Research 112:331–344. https://doi.org/10.1016/j.mycres.2007.10.014

Holdenrieder O, Greig B (1998) Biological methods of control. In: *Heterobasidion annosum*, Biology, Ecology, Impact and Control. CAB International, Wallingford, pp 235–258

Howell CR (2003) Mechanisms Employed by *Trichoderma* Species in the Biological Control of Plant Diseases: The History and Evolution of Current Concepts. Plant Disease 87:4–10. https://doi.org/10.1094/PDIS.2003.87.1.4

Hulme PE (2021) Unwelcome exchange: International trade as a direct and indirect driver of biological invasions worldwide. One Earth 4:666–679. https://doi.org/10.1016/j.oneear.2021.04.015

Hyde KD, Saleh A, Aumentado HDR, et al (2024) Fungal numbers: global needs for a realistic assessment. Fungal Diversity. https://doi.org/10.1007/s13225-024-00545-8

Hyde KD, Soytong K (2008) The fungal endophyte dilemma. Fungal Diversity 33:163–173.

International Biocontrol Manufacturers Association (2025) Definition: Bioprotection as the global term for all biocontrol technologies.

Jurc D, Ogris N, Slippers B, Stenlid J (2006) First report of eutypella canker of *Acer pseudoplatanus* in Europe. Plant Pathology 55:577–577. https://doi.org/10.1111/j.1365-3059.2006.01426.x

Karadžić D, Stanivuković Z, Milanović S, et al (2020) Development of *Neonectria punicea* pathogenic symptoms in juvenile *Fraxinus excelsior* trees. Front Plant Sci 11:592260. https://doi.org/10.3389/fpls.2020.592260

Kehr R (1998) Zur Bedeutung pilzlicher Endophyten bei Waldbäumen. In: Wulf A, Biologische Bundesanstalt für Land- und Forstwirtschaft in Berlin und Braunschweig (eds) 100 Jahre Pflanzenschutzforschung: Aktuelle Forschungsschwerpunkte im Forst- und Rebschutz: = 100 years research in plant protection: aspects of current research in forest and grapevine protection. Parey, Berlin, pp 8–30.

Kehr RD, Wulf A (2009) Diseases, Disorders and Pests of Selected Valuable Broadleaved Tree Species. In: Valuable Broadleaved Forests in Europe. Brill, Leiden, Boston, Köln, pp 61–84

Keller NP, Turner G, Bennett JW (2005) Fungal secondary metabolism — from biochemistry to genomics. Nat Rev Microbiol 3:937–947. https://doi.org/10.1038/nrmicro1286

Kelnarová I, Černý K, Zahradník D, Koukol O (2017) Widespread latent infection of *Cryptostroma corticale* in asymptomatic *Acer pseudoplatanus* as a risk for urban plantations. Forest Pathology 47:e12344. https://doi.org/10.1111/efp.12344

Kenis M, Hurley BP, Colombari F, et al (2019) Guide to the classical biological control of insect pests in planted and natural forests. FAO Forestry Paper No. 182. Rome, FAO.

Keswani C, Singh HB, Hermosa R, et al (2019) Antimicrobial secondary metabolites from agriculturally important fungi as next biocontrol agents. Appl Microbiol Biotechnol 103:9287–9303. https://doi.org/10.1007/s00253-019-10209-2

Kirisits T (2007) Die *Petrakia*-Blattbräune des Bergahorns. Forstschutz Aktuell 40:28–31.

Koukol O, Kelnarová I, Černý K (2014) Recent observations of sooty bark disease of sycamore maple in Prague (Czech Republic) and the phylogenetic placement of *Cryptostroma corticale*. Forest Pathology 45:21–27. https://doi.org/10.1111/efp.12129

Kowalski T, Kehr RD (1992) Endophytic fungal colonization of branch bases in several forest tree species. Sydowia 44:137–168

Kowarik I (2023) The Mediterranean tree *Acer monspessulanum* invades urban greenspaces in Berlin. Dendrobiology 89:20–26. https://doi.org/10.12657/denbio.089.002

Krabel D, Wolf H (2013) Sycamore Maple (*Acer pseudoplatanus* L.). In: Pâques LE (ed) Forest Tree Breeding in Europe. Springer Netherlands, Dordrecht, pp 373–402

Lacap DC, Hyde KD, Liew ECY (2003) An evaluation of the fungal ‘morphotype’ concept based on ribosomal DNA sequences. Fungal Diversity 12:53–66

Lahlali R, Peng G (2014) Suppression of clubroot by *Clonostachys rosea* via antibiosis and induced host resistance. Plant Pathology 63:447–455. https://doi.org/10.1111/ppa.12112

Langer GJ, Bressem U, Habermann M (2013) Vermehrt Pilzkrankheiten an Bergahorn in Nordwestdeutschland. AFZ-Der Wald 6:22–26

Langer GJ, Bußkamp J, Terhonen E, Blumenstein K (2021) Fungi inhabiting woody tree tissues. In: Forest Microbiology. Elsevier, pp 175–205

Langer GJ, Peters S, Bußkamp J, Bien S (2024) *Cryptostroma corticale* and fungal endophytes associated with *Fraxinus excelsior* affected by ash dieback. J Plant Dis Prot 131:1289–1299. https://doi.org/10.1007/s41348-023-00750-8

Large M, Galbraith M (2017) Implications for selected indigenous fauna of Tiritiri Matangi of the establishment of *Austropuccinia psidii* (G. Winter) Beenken (myrtle rust) in northern New Zealand. Perspectives in Biosecurity 2 2:6–26.

Lehtijärvi A, Oskay F, Doğmuş Lehtijärvi HT, et al (2018) *Ceratocystis platani* is killing plane trees in Istanbul (Turkey). Forest Pathology 48:e12375. https://doi.org/10.1111/efp.12375

Leuschner C, Ellenberg H (2017) Ecology of Central European Forests: Vegetation Ecology of Central Europe, Volume I. Springer International Publishing, Cham.

Lorenc F (2024) *Cryptostroma corticale* and its relationship to other pathogens and pests on *Acer pseudoplatanus*. Journal of Forest Science. https://doi.org/10.17221/41/2024-JFS

Luchi N, Ghelardini L, Belbahri L, et al (2013) Rapid Detection of *Ceratocystis platani* Inoculum by Quantitative Real-Time PCR Assay. Appl Environ Microbiol 79:5394–5404. https://doi.org/10.1128/AEM.01484-13

Luchi N, Ioos R, Santini A (2020) Fast and reliable molecular methods to detect fungal pathogens in woody plants. Appl Microbiol Biotechnol 104:2453–2468. https://doi.org/10.1007/s00253-020-10395-4

Maherali H, Klironomos JN (2007) Influence of Phylogeny on Fungal Community Assembly and Ecosystem Functioning. Science 316:1746–1748. https://doi.org/10.1126/science.1143082

Martínez-Álvarez P, Rodríguez-Ceinós S, Martín-García J, Diez JJ (2012) Monitoring endophyte populations in pine plantations and native oak forests in Northern Spain. Forest Syst 21:373–382. https://doi.org/10.5424/fs/2012213-02254

Martínez-Arias C, Sobrino-Plata J, Gil L, et al (2021) Priming of Plant Defenses against *Ophiostoma novo-ulmi* by Elm (*Ulmus minor* Mill.) Fungal Endophytes. JoF 7:687. https://doi.org/10.3390/jof7090687

Mckinney L, Nielsen L, Collinge D, et al (2014) The ash dieback crisis: Genetic variation in resistance can prove a long-term solution. Plant Pathology 63:. https://doi.org/10.1111/ppa.12196

McMullin DR, Green BD, Prince NC, et al (2017) Natural Products of *Picea* Endophytes from the Acadian Forest. J Nat Prod 80:1475–1483. https://doi.org/10.1021/acs.jnatprod.6b01157

Metzler B (2006) *Cryptostroma corticale* an Bergahorn nach dem Trockenjahr 2003. Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft 400:161–162.

Migliorini D, Auger-Rozenberg M-A, Battisti A, et al (2023) Towards a global sentinel plants research strategy to prevent new introductions of non-native pests and pathogens in forests. The experience of HOMED. RIO 9:e96744. https://doi.org/10.3897/rio.9.e96744

Ministero delle Politiche Agricole Alimentari e Forestali (2015) DM 6 luglio 2015

Moreau C, Moreau M (1951) La ‚Suie‘ des Sycamores a Paris. Bulletin de la Société mycologique de France 67:404–418

Muggia L, Kopun T, Grube M (2017) Effects of growth media on the diversity of culturable fungi from lichens. Molecules 22:824. https://doi.org/10.3390/molecules22050824

Muller E, Dvořák M, Marçais B, et al (2023) Conditions of emergence of the Sooty Bark Disease and aerobiology of *Cryptostroma corticale* in Europe. NB 84:319–347. https://doi.org/10.3897/neobiota.84.90549

Nawrot-Chorabik K, Sułkowska M, Gumulak N (2022) Secondary Metabolites Produced by Trees and Fungi: Achievements So Far and Challenges Remaining. Forests 13:1338. https://doi.org/10.3390/f13081338

Nielsen KF, Holm G, Uttrup LP, Nielsen PA (2004) Mould growth on building materials under low water activities. Influence of humidity and temperature on fungal growth and secondary metabolism. International Biodeterioration & Biodegradation 54:325–336. https://doi.org/10.1016/j.ibiod.2004.05.002

Niskanen T, Lücking R, Dahlberg A, et al (2023) Pushing the Frontiers of Biodiversity Research: Unveiling the Global Diversity, Distribution, and Conservation of Fungi. Annu Rev Environ Resour 48:149–176. https://doi.org/10.1146/annurev-environ-112621-090937

Nordwestdeutsche Forstliche Versuchsanstalt, Hessisches Ministerium für Landwirtschaft und Umwelt, Weinbau, Forsten, Jagd und Heimat (2024) Waldzustandsbericht 2024 für Hessen. Zendo. https://doi.org/10.5281/zenodo.13865674

Ocasio-Morales RG, Tsopelas P, Harrington TC (2007) Origin of *Ceratocystis platani* on Native *Platanus orientalis* in Greece and Its Impact on Natural Forests. Plant Disease 91:901–904. https://doi.org/10.1094/PDIS-91-7-0901

Oliveira Longa CM, Vai N, Maresi G (2016) *Cryptostroma corticale* in the northern Apennines (Italy). Phytopathologia Mediterranea 55:136–138. https://doi.org/10.14601/Phytopathol_Mediterr-17164

Oses R, Valenzuela S, Freer J, et al (2008) Fungal endophytes in xylem of healthy Chilean trees and their possible role in early wood decay. Fungal Diversity 33:77–86

Paoletti M, Saupe SJ, Clavé C (2007) Genesis of a Fungal Non-Self Recognition Repertoire. PLoS ONE 2:e283. https://doi.org/10.1371/journal.pone.0000283

Pasta S, de Rigo D, Caudullo G (2016) *Acer pseudoplatanus* in Europe: distribution, habitat, usage and threats. In: San-Miguel-Avanz J, de Rigo D, Caudullo G, et al. (eds) The European Atlas of Forest Tree Species: modelling, data and information on forest tree species. Publ. Off. EU, Luxembourg, p e01665a+.

Pautasso M, Dehnen-Schmutz K, Holdenrieder O, et al (2010) Plant health and global change – some implications for landscape management. Biological Reviews 85:729–755. https://doi.org/10.1111/j.1469-185X.2010.00123.x

Pearce RB (2000) Decay development and its restriction in trees. Journal of Aroboriculture 1–10.

Peršoh D, Melcher M, Flessa F, Rambold G (2010) First fungal community analyses of endophytic ascomycetes associated with *Viscum album* ssp. *austriacum* and its host *Pinus sylvestris*. Fungal Biology 114:585–596. https://doi.org/10.1016/j.funbio.2010.04.009

Peters S, Fuchs S, Bien S, et al (2023) Fungi associated with stem collar necroses of *Fraxinus excelsior* affected by ash dieback. Mycol Progress 22:52. https://doi.org/10.1007/s11557-023-01897-2

Petrini O (1991) Fungal Endophytes of Tree Leaves. In: Andrews HJ, Hirano SS (eds) Microbial Ecology of Leaves. Springer-Verlag, New York and Berlin, pp 179–197.

Petrini O, Fisher PJ (1988) A comparative study of fungal endophytes in xylem and whole stem of *Pinus sylvestris* and *Fagus sylvatica*. Transactions of the British Mycological Society 91:233–238. https://doi.org/10.1016/S0007-1536(88)80210-9

Plate HP, Schneider R (1965) Ein Fall von asthmaartiger Allergie, verursacht durch den Pilz *Cryptostroma corticale*. Nachrichtenblatt des deutschen Pflanzenschutzdienstes 17:100–101.

Pratt JE, Gibbs JN, Webber JF (1999) Registration of *Phlebiopsis gigantea* as a Forest Biocontrol Agent in the UK: Recent Experience. Biocontrol Science and Technology 9:113–118. https://doi.org/10.1080/09583159929974

Prospero S, Botella L, Santini A, Robin C (2021) Biological control of emerging forest diseases: How can we move from dreams to reality? Forest Ecology and Management 496:119377. https://doi.org/10.1016/j.foreco.2021.119377

Przybył K (2002) Fungi associated with necrotic apical parts of *Fraxinus excelsior* shoots. Forest Pathology 32:387–394. https://doi.org/10.1046/j.1439-0329.2002.00301.x

Qaderi MM, Martel AB, Strugnell CA (2023) Environmental Factors Regulate Plant Secondary Metabolites. Plants 12:447. https://doi.org/10.3390/plants12030447

Rabbee MF, Ali MdS, Islam MdN, et al (2024) Endophyte mediated biocontrol mechanisms of phytopathogens in agriculture. Research in Microbiology 104229. https://doi.org/10.1016/j.resmic.2024.104229

Rabiey M, Hailey LE, Roy SR, et al (2019) Endophytes vs tree pathogens and pests: can they be used as biological control agents to improve tree health? Eur J Plant Pathol 155:711–729. https://doi.org/10.1007/s10658-019-01814-y

Raffa KF, Brockerhoff EG, Grégoire J-C, et al (2023) Approaches to Forecasting Damage by Invasive Forest Insects and Pathogens: A Cross-Assessment. BioScience 73:85–111. https://doi.org/10.1093/biosci/biac108

Ragazzi A, Moricca S, Capretti P, et al (2003) Differences in composition of endophytic mycobiota in twigs and leaves of healthy and declining *Quercus* species in Italy. Forest Pathol 33:31–38. https://doi.org/10.1046/j.1439-0329.2003.3062003.x

Redman RS, Freeman S, Clifton DR, et al (1999) Biochemical Analysis of Plant Protection Afforded by a Nonpathogenic Endophytic Mutant of *Colletotrichum magna*. Plant Physiology 119:795–804. https://doi.org/10.1104/pp.119.2.795

Reshi ZA, Ahmad W, Lukatkin AS, Javed SB (2023) From Nature to Lab: A Review of Secondary Metabolite Biosynthetic Pathways, Environmental Influences, and *In Vitro* Approaches. Metabolites 13:895. https://doi.org/10.3390/metabo13080895

Rigling D, Prospero S (2018) *Cryphonectria parasitica*, the causal agent of chestnut blight: invasion history, population biology and disease control. Mol Plant Pathol 19:7–20. https://doi.org/10.1111/mpp.12542

Robeck P, Heinrich R, Schumacher J, et al (2008) Status der Rußrindenkrankheit des Ahorns in Deutschland. In: Jahrbuch der Baumpflege. Haymarket Media GmbH, Braunschweig, pp 238–244.

Rodriguez RJ, Redman RS, Henson JM (2004) The Role of Fungal Symbioses in the Adaptation of Plants to High Stress Environments. Mitigation and Adaptation Strategies for Global Change 9:261–272. https://doi.org/10.1023/B:MITI.0000029922.31110.97

Rodriguez RJ, White Jr JF, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytologist 182:314–330. https://doi.org/10.1111/j.1469-8137.2009.02773.x

Rożek K, Chmolowska D, Odriozola I, et al (2023) Soil fungal and bacterial community structure in monocultures of fourteen tree species of the temperate zone. Forest Ecology and Management 530:120751. https://doi.org/10.1016/j.foreco.2022.120751

Runge F (1994) Die Pflanzengesellschaften Mitteleuropas, 12/13 edn. Aschendorff Verlag, Münster (Westfalen).

Rungjindamai N, Jones EBG (2024) Why Are There So Few *Basidiomycota* and Basal Fungi as Endophytes? A Review. JoF 10:67. https://doi.org/10.3390/jof10010067

Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998) Fungal endophytes: A continuum of interactions with host plants. Annual Review of Ecology and Systematics 29:319–343.

Samuels GJ, Pardo-Schultheiss R, Hebbar KP, et al (2000) *Trichoderma stromaticum* sp. nov., a parasite of the cacao witches broom pathogen. Mycological Research 104:760–764. https://doi.org/10.1017/S0953756299001938

Sanchez S, Demain AL (2011) Scientific Fundamentals of Biotechnology. In: Moo-Young M (ed) Comprehensive biotechnology, 3rd ed. Elsevier, Amsterdam; Boston.

Santini A, Ghelardini L, De Pace C, et al (2013) Biogeographical patterns and determinants of invasion by forest pathogens in Europe. New Phytologist 197:238–250. https://doi.org/10.1111/j.1469-8137.2012.04364.x

Sanz-Ros AV, Müller MM, San Martín R, Diez JJ (2015) Fungal endophytic communities on twigs of fast and slow growing Scots pine (*Pinus sylvestris* L.) in northern Spain. Fungal Biology 119:870–883. https://doi.org/10.1016/j.funbio.2015.06.008

Schlegel M, Queloz V, Sieber TN (2018) The Endophytic Mycobiome of European Ash and Sycamore Maple Leaves – Geographic Patterns, Host Specificity and Influence of Ash Dieback. Front Microbiol 9:2345. https://doi.org/10.3389/fmicb.2018.02345

Schlößer R, Bien S, Bußkamp J, et al (2025) On the lookout for a potential antagonist against *Cryptostroma corticale*-an insight through *in vitro* dual culture studies. Frontiers in Forests and Global Change 7: 1518972. https://doi.org/10.3389/ffgc.2024.1518972

Schlößer R, Bien S, Langer GJ, Langer EJ (2023) Fungi associated with woody tissues of *Acer pseudoplatanus* in forest stands with different health status concerning sooty bark disease (*Cryptostroma corticale*). Mycol Progress 22:13. https://doi.org/10.1007/s11557-022-01861-6

Schlößer R, Santini A, Pepori AL, et al (2024) *Cryptostroma corticale* in Italy: new reports of sooty bark of *Acer pseudoplatanus* and first outbreak on *Acer campestre*. Phytopathologia Mediterranea 63:399–406. https://doi.org/10.36253/phyto-15744

Schmidt O, Roloff A (2014) *Acer pseudoplatanus*. In: Stimm B, Roloff A, Lang UM, Weisgerber H (eds) In Enzyklopädie der Holzgewächse: Handbuch und Atlas der Dendrologie.

Schneidewind A (2005) Untersuchungen zur Standorteignung von *Acer pseudoplatanus* L. als Straßenbaum in Mitteldeutschland unter besonderer Berücksichtigung abiotischer und biotischer Stressfaktoren. Tenea, Bristol.

Schulz B, Boyle C (2005) The endophytic continuum. Mycological Research 109:661–686. https://doi.org/10.1017/S095375620500273X

Schulz B, Römmert A-K, Dammann U, et al (1999) The endophyte-host interaction: a balanced antagonism? Mycological Research 103:1275–1283. https://doi.org/10.1017/S0953756299008540

Schulz B, Sucker J, Aust HJ, et al (1995) Biologically active secondary metabolites of endophytic *Pezicula* species. Mycological Research 99:1007–1015. https://doi.org/10.1016/S0953-7562(09)80766-1

Schumacher J, Leonhard S, Wulf A, Heydeck P (2006) Die Schwarze Buchenkohlenbeere als Parasit und Holzfäuleerreger an Rotbuchen - ein Doppelgänger des Brandkrustenpilzes? Forst und Holz 61:369–372.

Schwarze FWMR (2018) Diagnose und Prognose der Fäuledynamik in Stadtbäumen. MycoSolutions AG, St. Gallen.

Schwerdtfeger F (1981) Die Waldkrankheiten: ein Lehrbuch der Forstpathologie und des Forstschutzes, 4., neubearb. Aufl. Parey, Hamburg.

Seyedsayamdost MR (2019) Toward a global picture of bacterial secondary metabolism. Journal of Industrial Microbiology and Biotechnology 46:301–311. https://doi.org/10.1007/s10295-019-02136-y

Sicard A, Zeilinger AR, Vanhove M, et al (2018) *Xylella fastidiosa* : Insights into an Emerging Plant Pathogen. Annu Rev Phytopathol 56:181–202. https://doi.org/10.1146/annurev-phyto-080417-045849

Sieber TN (2007) Endophytic fungi in forest trees: are they mutualists? Fungal Biology Reviews 21:75–89. https://doi.org/10.1016/j.fbr.2007.05.004

Sinclair WA, Lyon HH (2005) Diseases of Trees and Shrubs - Second Edition. Comstock Publishing.

Singh BK, Delgado-Baquerizo M, Egidi E, et al (2023) Climate change impacts on plant pathogens, food security and paths forward. Nat Rev Microbiol 21:640–656. https://doi.org/10.1038/s41579-023-00900-7

Šnajdr J, Dobiášová P, Větrovský T, et al (2011) Saprotrophic basidiomycete mycelia and their interspecific interactions affect the spatial distribution of extracellular enzymes in soil: Saprotrophic basidiomycetes affect soil enzyme distribution. FEMS Microbiology Ecology 78:80–90. https://doi.org/10.1111/j.1574-6941.2011.01123.x

Stenberg JA, Sundh I, Becher PG, et al (2021) When is it biological control? A framework of definitions, mechanisms, and classifications. J Pest Sci 94:665–676. https://doi.org/10.1007/s10340-021-01354-7

Stone J, Petrini O (1997) Endophytes of Forest Trees: a Model for Fungus-Plant Interactions. In: Carroll GC, Tudzynski P (eds) Plant Relationships Part B. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 129–140.

Stone JK, Polishook JD, White Jr JF (2004) Endophytic Fungi. In: Biodiversity of fungi: inventory and monitoring methods. Elsevier, Amsterdam, pp 241–270.

Tanney JB, Feau N, Shamoun SF, et al (2024) *Cryptostroma corticale* (Ellis & Everh.) P. H. Greg. & S. Waller causing sooty bark disease in British Columbia, Canada. Canadian Journal of Plant Pathology 46:596–610. https://doi.org/10.1080/07060661.2024.2369324

Teoh ES (2016) Secondary Metabolites of Plants. In: Medicinal Orchids of Asia. Springer International Publishing, Cham, pp 59–73.

Terhonen E, Blumenstein K, Kovalchuk A, Asiegbu FO (2019) Forest Tree Microbiomes and Associated Fungal Endophytes: Functional Roles and Impact on Forest Health. Forests 10:42. https://doi.org/10.3390/f10010042

Thambugala KM, Daranagama DA, Phillips AJL, et al (2020) Fungi vs. Fungi in Biocontrol: An Overview of Fungal Antagonists Applied Against Fungal Plant Pathogens. Front Cell Infect Microbiol 10:604923. https://doi.org/10.3389/fcimb.2020.604923

Townrow JA (1953) The Biology of *Cryptostroma corticale* and the Sooty Bark Disease of Sycamore. In: Report on Forest Research. London, pp 118–120.

Unterseher M (2007) Fungal Biodiversity Research – from cultivation of foliar tree endophytes to the combination of ecological theory and next generation sequencing. Dissertation, Ernst Moritz Arndt Universität Greifswald.

Unterseher M, Otto P, Morawetz W (2005) Species richness and substrate specificity of lignicolous fungi in the canopy of a temperate, mixed deciduous forest. Mycol Progress 4:117–132. https://doi.org/10.1007/s11557-006-0115-7

Van Wees SC, Van Der Ent S, Pieterse CM (2008) Plant immune responses triggered by beneficial microbes. Current Opinion in Plant Biology 11:443–448. https://doi.org/10.1016/j.pbi.2008.05.005

Vandenkoornhuyse P, Quaiser A, Duhamel M, et al (2015) The importance of the microbiome of the plant holobiont. New Phytologist 206:1196–1206. https://doi.org/10.1111/nph.13312

Voglmayr H, Jaklitsch WM (2008) *Prosthecium* species with *Stegonsporium* anamorphs on *Acer*. Mycological Research 112:885–905. https://doi.org/10.1016/j.mycres.2008.01.020

Vogt J, Gillner S, Hofmann M, et al (2017) Citree: A database supporting tree selection for urban areas in temperate climate. Landscape and Urban Planning 157:14–25. https://doi.org/10.1016/j.landurbplan.2016.06.005

Vu T, Sikora R, Hauschild R (2006) *Fusarium oxysporum* endophytes induced systemic resistance against *Radopholus similis* on banana. Nematol 8:847–852. https://doi.org/10.1163/156854106779799259

Wang Q, Xu L (2012) Beauvericin, a Bioactive Compound Produced by Fungi: A Short Review. Molecules 17:2367–2377. https://doi.org/10.3390/molecules17032367

Westphal MI, Browne M, MacKinnon K, Noble I (2008) The link between international trade and the global distribution of invasive alien species. Biol Invasions 10:391–398. https://doi.org/10.1007/s10530-007-9138-5

Woodward S, Boddy L (2008) Chapter 7 Interactions between saprotrophic fungi. In: British Mycological Society Symposia Series. Academic Press, pp 125–141.

World Flora Online (2024) *Acer pseudoplatanus* L. In: World Flora Online (WFO). https://www.worldfloraonline.org/taxon/wfo-0000514908

Worrall JJ, Anagnost SE, Zabel RA (1997) Comparison of wood decay among diverse lignicolous fungi. Mycologia 89:199–219. https://doi.org/10.1080/00275514.1997.12026772

Wulf A, Leonhard S, Schumacher J (2005) Pilzkrankheiten an Bergahorn. LWF Wissen 62:41–44.

Yasanthika W, Wanasinghe D, Mortimer P, et al (2022) The importance of culture-based techniques in the genomic era for assessing the taxonomy and diversity of soil fungi. Mycosphere 13:724–751. https://doi.org/10.5943/mycosphere/13/1/8

Young CWT (1978) Sooty Bark Disease of Sycamore. Stationery Office Books, Department of Environment, London u.a.

Zhao Y, Chen X, Cheng J, et al (2022) Application of *Trichoderma* Hz36 and Hk37 as Biocontrol Agents against Clubroot Caused by *Plasmodiophora brassicae*. JoF 8:777. https://doi.org/10.3390/jof8080777

Appendix

Declaration of own contributions to the publications

Manuscript I

Sampling	Rebekka Schlößer, Steffen Bien
Labwork	Rebekka Schlößer, Steffen Bien
Analysis	Rebekka Schlößer
Manuscript work	
First draft	Rebekka Schlößer
Editing	Steffen Bien, Johanna Bußkamp, Gitta Jutta Langer, Ewald Johannes Langer

Manuscript II

Labwork	Rebekka Schlößer
Analysis	Rebekka Schlößer
Manuscript work	
First draft	Rebekka Schlößer
Editing	Steffen Bien, Johanna Bußkamp, Gitta Jutta Langer, Ewald Johannes Langer

The declarations must be confirmed in writing by the co-authors:

I hereby confirm the declarations of Ms. Rebekka Schlößer regarding the contributions:

Name:	Signature:
Steffen Bien	
Johanna Bußkamp	
Gitta Jutta Langer	
Ewald Johannes Langer	

Additional publications

In connection to the research project of this doctoral thesis in chronological order:

Schlößer R., Gawehn P., Bußkamp J., Langer G. (2020): Rußrindenkrankheit an Ahorn in hessischen Wäldern als Folge der Klimaerwärmung. Im Dialog (3): 8–10.

Schlößer R, Langer G (2021) Verbreitung der Rußrindenkrankheit des Ahorns in Deutschland. AFZ - Der Wald 24:28–32